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MYELIN STRUCTURES IN LIPOIDS AND THEIR RELATION TO ELECTRIC CHARGES.

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In a paper by Dr. G. W. Crile (1), reference is made to the myelin structures which grow out from lipoid films covered with various solutions. It was observed that the application of electrical polarization increased the growth of these myelin structures. In this paper further studies are published with the aim of correlating the growth of myelin structures with other physical properties.

The formation of myelin structures was first described by Virchow (2) in 1854. Haeckel (3) assigned an important role to these structures in vital phenomena; Lehmann (4) and Rhumbler (5) made further experiments. Recently Leathes (6) investigated the myelin structures formed by various lipoids. He took moving pictures of their growth and influenced the rate of growth by applying various salt solutions in different concentrations. It appeared that concentrated salt solutions retarded growth, while more dilute solutions stimulated it. Calcium salts inhibited growth. Various types of structures were obtained in solutions containing gelatin, egg white, serum hemoglobin, etc., and with mixtures of lecithin and cholesterol.

In our own investigations an attempt was made to bring this phenomenon into relationship with the electrical surface charge of thin films of lipoids. The basis for expecting that such a relationship is possible is the known fact that in thin lipoid films an orientation of the fatty acid molecules takes place (Leathes (7) and Adam (8)). The fatty acid group is attracted toward the water, while the methyl group of the paraffin chain is directed away from it. The fatty acid chains are arranged

parallel to each other, each lecithin molecule for instance occupying a surface of 56 square Å, according to Leathes' measurements on thin films spread over water. Trillat (9) found a similar parallel arrangement of fatty acid molecules with the Hull-Debye-Scherrer x-ray method. On films spread over metals he found that the distance between the lecithin molecules averaged 47 Å, which is a somewhat higher value than for films spread over water. Further indications of the polar arrangement of lipoid molecules are shown by the model experiments of Runnstorm (10) in which the molecules show double refraction and striated lamellae under the polarization microscope.

Actual measurements of the surface charge of cholesterol sols were made by Remesow (11) by the cataphoresis method, which gave interesting results; this method, however, could not be applied to lecithin emulsions.

In seeking a possible explanation for the formation of myelin structures the following observation is of value. The myelin structure is always surrounded by a thin film (Fig. 2B) while the inside of the structure is filled with liquid. In a dry particle of lipoid the fatty acid chains are necessarily orientated at random. If this particle is then surrounded with pure water, an immediate rearrangement of the fatty acid chain takes place in such a way that the acid ends point toward the water, while the paraffin ends point toward the inside of the particle. Water passes through between the orientated fatty acid chains, and consequently the surface of the lipoid particle begins to enlarge, long myelin structures rising out of the lipoid. The growth velocity of the myelin fibers must necessarily depend on the velocity with which the water penetrates through the surface film and increases it. This phenomenon might be explained as being due to various physical causes, like diffusion, swelling (similar to that of proteins), osmosis, surface tension, etc. This will be discussed later.

EXPERIMENTAL METHOD.

To avoid the difficulty of working with lecithin an ether extract of calf's brain was used. The tissue was thoroughly minced, rapidly dried at a temperature not exceeding 40° C. (higher temperatures lessen or inhibit the growth of myelin structures). The dried tissue was pulverized, extracted with ether, and filtered several times. This solution can be kept

for several weeks. Extracts which were over a month old were not used. The concentration of the ether solution was determined by evaporating a known amount of the solution to dryness under vacuum. Generally the ether solution was so diluted as to contain one per cent lipoid.

The growth of the myelin structures was observed in the following way. A well slide (similar to those used in tissue cultures) was thoroughly cleaned and a drop of the ether solution was deposited on it by means of a glass rod. The drop was of such dimensions that it corresponded to 0.1 cc. of the one per cent lipoid solution, containing therefore about 1 mg. of lipoid. The drop was deposited so that it formed a round, thin film about 5–7 mm. in diameter. The ether evaporated very rapidly, leaving a very thin film, which was generally somewhat thicker around the edge. This film was covered with several drops of the solution, the effect of which was to be tested, and a cover glass was placed over it. Slight pressure on the cover glass expelled the excess of liquid, which was blotted off, leaving a preparation which without drying could be observed over a period of several days. Vaseline or other sealing material was found to be unnecessary.

On account of the thinness of the film, the growth of myelin structures could be observed not only on the edge of the film, but throughout its surface. The standard comparison of growth was made with the help of a solution which contained the salts found in the brain dissolved in distilled water in the same proportion as they are present in the brain. The composition of 1000 cc. of this "brain-salt" solution was as follows: KCl—2.33 gm., Na_3PO_4—2.21 gm., K_3PO_4—0.89 gm., Na_2CO_3—0.11 gm., K_2SO_4—0.24 gm., $CaCl_2$—0.026 gm., $MgCl_2$—0.55 gm., distilled water to make 1000 cc. The calcium and magnesium salts formed a precipitate with the phosphate, which was filtered off. This solution was alkaline—pH 11.4. Myelin structures were readily formed in this solution. Immediately after a lipoid film was covered with this solution, small myelin growths appeared, not only on the edge of the film, but all over its surface. With a magnification of 600 the growth of the structures could be observed and with the aid of an ocular micrometer the growth could be readily followed.

In order to compare the effects of solutions of various salts in distilled water, a number of well slides were covered with the thin lipoid film; the various solutions were superimposed

as quickly as possible and the growth of the myelin structures was observed under the microscope at intervals of 5, 10 and 30 minutes. The results are given in Table I in which the best growths (that is, one comparable to that in the brain-salt solution) are indicated by xxx, medium growths by xx, slight growths by x and no growth at all by —.

TABLE I.

GROWTH OF MYELIN STRUCTURES IN VARIOUS SOLUTIONS.

SOLUTION	CONCENTRATION OF SOLUTION IN MOLS.					
	1	0.1	0.01	0.001	0.0001	0.00001
NaCl	—	x	xx	xxx	xxx	xx
KCl	—	x	xx	xx	xx	xx
$CaCl_2$	—	—	—	x	xx	xx
$MgCl_2$	—	—	—	x	xx	xx
$CuCl_2$	—	—	—	x	xx	xx
$FeCl_3$	—	—	—	—	x	xx
HCl	—	—	—	—	x	x
NaOH	x	xx	xx	xxx	xx	xx
K_3PO_4	x	xx	xx	xxx	xxx	xx
K_2SO_4	x	xx	xxx	xx	x	x
KCN	x	xx	xxx	xx	x	x
NaI	x	x	xx	x	x	x
Na_2CO_3	x	xx	xxx	xx	x	x

In solutions of the chlorides the growth increased in direct relation to the degree of dilution. The bivalent salts inhibited growth in 0.01 molar solutions and the trivalent $FeCl_3$ inhibited growth even in 0.001 molar solutions. No growth was observed in acid solutions except in those in which the pH was above 3.6, while NaOH and K_3PO_4 favored growth even in higher concentrations. Anions such as SO_4, CN, CO_2 seemed to favor growth as compared with Cl.

The characteristic growths produced by different concentrations are indicated in Fig. 1, which shows the effect of (A) n, (B) 0.01n and (C) 0.001n NaCl solutions on the growth of myelin structures; while Table I gives the approximate grades of growth (—, x, xx, and xxx, respectively). Non-electrolytes have a marked influence on growth. For instance, an xx growth appears in a 10 per cent solution, glucose and even a saturated solution of glucose is not able to stop growth. Various dilutions of ethyl alcohol also exhibit a marked influence.

FIGURE 1.

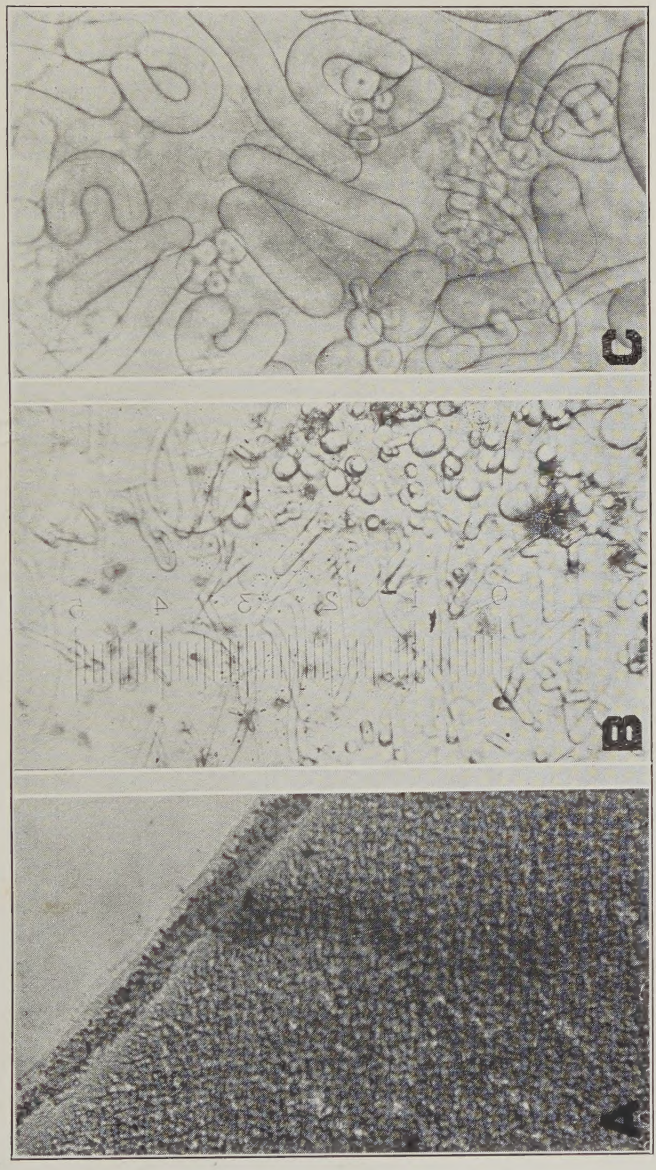

FIG. 1. Photomicrographs showing the effect of NaCl solutions on the growth of myelin structures.
A: n NaCl, growth—cataphoretical charge, 0 millivolt.
B: 0.1 n NaCl, xx, cataphoretical charge, —51.
C: 0.001 n NaCl, xxx, cataphoretical charge, —87.

In mixtures of salt solutions an antagonistic effect can be observed. If a growth-inhibiting solution of $CaCl_2$ is mixed with a growth-favoring solution, a fair growth of structures can be seen. In order to compare the antagonistic effects of various solutions, a further series of observations was made by dissolving the various agents in the "brain-salt" solution instead of in distilled water (Table II). In this way the action

TABLE II.

GROWTH OF MYELIN STRUCTURES IN A MIXTURE OF VARIOUS SOLUTIONS WITH THE BRAIN SALT SOLUTION.

SOLUTION	CONCENTRATION OF SOLUTION IN MOLS.				
	1	0.1	0.01	0.001	0.0001
NaCl	x	xx	xxx	xxx	xxx
KCl	x	x	xx	xxx	xxx
$CaCl_2$	—	—	x	xx	xxx
$MgCl_2$	—	—	x	xx	xxx
$FeCl_3$	—	—	—	xxx	xxx
$AlCl_3$	—	—	—	xxx	xxx
NaI	xx	xx	xx	xxx	xxx
NaBr	xx	xxx	xxx	xx	xxx
	CONCENTRATION OF SOLUTION IN PER CENTS				
	1	0.1	0.01	0.001	0.0001
Adrenalin	.....	x	xxx	xx	xxx
Atropin	x	xx	xxx	.....	.....
Caffein	xxx	xxx	xxx	.....	.....
Luminal	xx	xx	xxx	.....	.....
Strychnin	x	xx	xx	xxx	.....
Ethyl alcohol	x	xxx	xx	xxx	.....
	50%	25%	10%	2.5%	.....
Formaldehyd	—	x	xx	xxx	.....
	40%	10%	1%	0.1%	.....
Glucose	x	xx	xxx	.....	.....
	20%	10%	1%	.....	.....

of the agents is observed in the same salt solution as that which is present in the brain, although it is in no way implied that in this way a comparison between the effect of agents on the lipoid emulsion and on the brain could be made directly.

Some of the metal chlorides form a precipitate with the phosphate, etc., which results in the removal of the added metal ions from the solution. As shown in Table II, however, the salts exert a marked influence although their inhibiting action

in the higher concentrations is counterbalanced by the "brain-salt" solution.

The effect of alcohol is shown in Fig. 2. The inhibiting effect of a 50 per cent solution (C), and the accelerating effect of a 25 per cent solution (B) is evident. The inhibiting effects of some of the other agents in relatively low concentrations are remarkable.

CATAPHORESIS MEASUREMENTS.

A slightly modified Northrop cataphoresis apparatus was used. The current was conducted through reversible electrodes. The charge of the particles was calculated from the Helmholtz-Schmoluchowski formula. With concentrated solutions the viscosity of the solution was also taken into account.

The emulsion was obtained by drying some lipoid thoroughly and mixing it with the solution to be tested. After mixing, the usual myelin formations grew out of the mass of lipoid and when the mixture was shaken for some time the longitudinal parts of the long myelin structures could be seen floating in the mixture. When the mixture was shaken vigorously, the myelin structures were broken up into oblong tubular particles which were filled with liquid. The length of these particles was generally about 20$\mu\mu$, although some larger particles were found occasionally. When the suspension was allowed to stand for several hours practically the whole of the lipoid was changed into such particles.

Another method for preparing emulsions was to mix the ether solution of the lipoid with distilled water or the salt solution to be tested. As much of the ether as possible was evaporated under reduced pressure at a temperature of 40° C. In this way a suspension of round globules was obtained, in which a rapid growth of myelin structures could be seen.

The emulsion of myelin structures was placed in the cataphoresis apparatus and the velocity of the particles in an electric field was measured with a stop watch. The necessary precautions in regard to differences in the charge in the capillary tube were taken by always measuring the velocity at the same level of the capillary; in this way all the observations could be compared. While there might be some theoretical objection to the use of the formula, as the charge was always calculated from the same formula only relative values were compared. Some authors prefer to give the cataphoretical velocities as measured directly.

FIG. 2. Photomicrographs showing the effect of ethyl alcohol solution on the growth of myelin structures.
A: 10 per cent solution growth xxx, cataphoretical charge, —62 millivolts.
B: 25 per cent solution xxx, cataphoretical charge, —75.
C: 50 per cent solution x, cataphoretical charge, —18.

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In Table III are given the charges in millivolts of myelin particles in the various solutions. The charge of the lipoid in distilled water alone is —78 millivolts. Very dilute salt solutions increase the charge, while concentrated salt solutions generally decrease the charge until the particles become discharged or their charge is even reversed. The best method of observation was to prepare a suspension in distilled water.

TABLE III.

GROWTH OF MYELIN STRUCTURES IN VARIOUS SOLUTIONS COMPARED WITH THEIR ELECTRIC CHARGE (MILLIVOLTS).

SOLUTION	CONCENTRATION OF SOLUTIONS IN MOLS.							
	1	0.5	0.1	0.01	0.001	0.0001	0.00001	H_2O
NaCl	—	—	x	xx	xxx	xxx	xx	.....
	0	—11	—51	—85	—87	.....	.....	.....
KCl	—	—	x	xx	xx	xx	xx	.....
	5	0	—43	—63	—73	.....	.....	.....
$CaCl_2$	—	—	—	—	x	xx	xx	.....
	7	0	0	—17	—49	.....	.....	.....
$CuCl_2$	—	—	—	—	x	xx	xx	xx
	.....	.....	.....	0	—46	—61	—70	—68
$FeCl_3$	—	—	—	—	—	x	xx	xx
	.....	0	38	66	0	—49	—51	—70
NaOH	x	xx	xx	xx	xxx	xx	xx	xx
	—12	.....	—48	—91	—108	—84	—89	—98
K_3PO_4	x	xx	xx	xx	xxx	xxx	xx	xx
	—15	.....	—36	—62	—111	—94	—78	—100
Ethyl alcohol	50%	25%	10%	1%	.....	.....	.....	.....
	x	xxx	xxx	xx	.....	.....	.....	.....
	—18	—75	—62	—43	.....	.....	.....	.....
Urethane	1%	0.1%	0.01%	0.001%	.....	.....	.....	.....
	—	x	x	—	.....	.....	.....	.....
	—17	—36	—47	—18	.....	.....	.....	.....

The cataphoresis cell was filled with the emulsion and the velocity of the particles determined; a sufficient amount of salt solution was then added, the emulsion was mixed in an attachment of the cataphoresis cell and the velocity determined again without removing the emulsion from the apparatus. In this way, successive concentrations of the same salt were determined for their effect in changing the electrical charge.

In distilled water the whole amount of the lipoid changes very quickly into myelin forms. If subsequently an agent is added which discharges the particles or reverses their charge, the small structures shrivel up or change into shapeless masses, the velocity of which may still be determined. On the other

hand, if the discharging agent is added directly to a compact mass of lipoid, no myelin formation occurs generally, and so it is impossible to measure the change of the charge.

The peculiar changes observed are similar to those found by Heesch (12), by Remesow (11) in observations on cholesterol particles and by Brown and Broom (13) in observations on bacteria. Nevertheless such changes are not characteristic of lipoid alone, as various other suspensions show similar changes in the charge during the influence of bivalent and trivalent salts. Obviously, a surface charge or a potential difference will not produce myelin structures in most substances, but naturally the conclusion will be drawn from Table III that *the growth of the myelin structures is proportional to their negative electrical charge,* in the solutions which were used in our experiments.

INFLUENCE OF GALVANIC CURRENT ON THE GROWTH OF MYELIN STRUCTURES ON A LIPOID FILM.

If the growth of myelin structures in various salt solutions is proportional to the electric charge or electric potential difference between the film and the salt solution, then it should be possible to increase this potential difference by applying a constant galvanic current through the film. In such a case an electric polarization will result on the film, which will act as an increased charge. The following experiment was conducted. A microscopic slide was equipped with two reversible electrodes (silver needles covered electrolytically with silver chloride) which were separated by an interval of about 1 cm. A small drop of lipoid was placed between the electrodes, and a few drops of "brain-salt" solution were superimposed, so that the solution covered the electrodes. An electric current of about 1.5 volts was then passed through the solution. The myelin structures immediately began to grow rapidly all over the drop of lipoid bending towards the positive pole of the applied current. The growth was compared with that observed in an entirely identical drop in an experiment which was made under the same conditions and at the same time, except that in the latter experiment the electrodes were omitted. B and C in Fig. 3 show the growth with and A without electrical polarization. It will be noted that the structures point directly towards the positive pole. These experiments show not only that the growth is proportional to the negative charge of the

FIGURE 3.

FIG. 3. Photomicrograph showing the effect of electrical polarization on the growth of myelin structures.
A: without polarization. B and C: with polarization.
Note the direction and bending in C of the myelin structures toward the positive pole of the applied current.

myelin structures in the various salt solutions, but also that in the same salt solution an artificially increased charge will produce a very much increased growth.

MEASUREMENTS OF SURFACE TENSION AND VISCOSITY.

The surface tension of the lipoid suspension was measured with Traube's stalagmometer and parallel measurements of viscosity were made with a capillary viscosimeter. The procedure was as follows: The viscosimeter was filled with the lipoid suspension (1 per cent lipoid in distilled water). The determinations were made, and the same sample was then transferred to the stalagmometer and surface tension readings were taken. The solution, the effect of which was to be tested, was then added to the suspension and the surface tension and viscosity determined again on the same sample.

TABLE IV.

GROWTH OF MYELIN STRUCTURES COMPARED WITH THE ELECTRIC CHARGE OF THE LIPOID AND THE SURFACE TENSION WITH AND VISCOSITY OF THE LIPOID EMULSION.

MATERIAL	RELATIVE SURFACE TENSION	RELATIVE VISCOSITY	ELECTRIC CHARGE MILLIVOLT	GROWTH
Lipoid suspension in distilled water.....	0.76	1.00	—98	xx
Same in 0.0001 n NaOH.....	0.89	1.08	—84	xx
Same in 0.001 n NaOH.....	0.93	1.00	—108	xxx
Same in 0.01 n NaOH.....	0.88	0.95	—91	xx
Same in 0.1 n NaOH.....	0.83	0.92	—48	xx
Same in 0.2 n NaOH.....	0.92	0.95	—40	xx

The relative values of the viscosity, surface tension, the electric charge in millivolts and the observed growth of myelin structures are compiled in Table IV. Values are given only for a lipoid suspension in NaOH solutions, as this is sufficient to illustrate that there is no relation between the surface tension or the viscosity and the growth of myelin structures. Other measurements were made with electrolytes and non-electrolytes.

DISCUSSION.

As was mentioned above, the basic explanation for the growth of myelin structures is that water actually penetrates through the lipoid-water interface. Such a penetration might be explained possibly by the presence of various physical changes like osmosis, diffusion, surface tension, etc. If this is true,

then the changes of the physical constants must result in a considerable difference between the effects of different solutions, as for example of NaCl and HCl, and furthermore the constants must change considerably with the dilution, as for example in n and in n/100 NaCl. It is evident that there is no proportionality between the physical properties of the solution and the growth of myelin structures.

SUMMARY.

1. The growth of myelin structures in lipoid extracts of mammalian brain was found to depend on the concentration of the solution. No myelin growth took place in concentrated solutions; with increasing dilution the growth reached a maximum and slightly decreased again with greater dilution.

2. Bivalent salts have a considerable inhibiting action even in n/100 molecular solutions, while trivalent salts inhibit growth even more.

3. An antagonistic action was found to take place in mixtures of salt solutions.

4. The growth of myelin structures is proportional to their negative electrical charge.

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DIGESTION IN BLOWFLY LARVAE, PHORMIA REGINA MEIGEN, USED IN THE TREATMENT OF OSTEOMYELITIS.*

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The role of digestion in blowfly larvae is closely associated with the use of these maggots in the treatment of osteomyelitis. It is interrelated with such factors as methods of aseptic rearing, food requirements, and the activities of these maggots within the wounds involving the success of the treatment. In this study emphasis was placed on the qualitative determination of enzymes present and their location in the digestive tract, the effect of bacteria on digestion, and the analysis of the excreta.

Many articles have been written about digestion in various flies, most of the investigators attempting to prove or disprove the necessity for the presence of bacteria in the food. Atkin and Bacot (1917) maintained that bacteria or yeast was necessary in the food of the mosquito *Stegomyia fasciata*. Baumberger (1919) showed that Drosophila could not be raised without live yeast. According to Bogdanow (1906 and 1908) Calliphorid larvae failed to develop in the absence of micro-organisms. Guyenot (1906, 1907, and 1917) stated that Lucilia lived on the liquid products of bacteria, were unable to produce digestive ferments, and that this was done for them by bacteria. On the other hand, Wollman (1911) maintained that Calliphorid larvae could be raised without the presence of bacteria. Glaser (1924) bred two generations of Drosophila on aseptic yeast, and Northrup (1926) succeeded in rearing 230 generations of Drosophila aseptically. Hobson (1932a) raised larvae of *Lucilia sericata* aseptically on autoclaved sheep's brain, but was not successful when he used types of muscle such as that found in a guinea pig or a cod (1932b).

* One of a series of research projects done as a part of the study upon the use of fly larvae in the treatment of osteomyelitis being made by the Department of Zoology and Entomology under the direction of D. F. Miller, in co-operation with the Departments of Medical and Surgical Research and of Orthopedic Surgery, in the Ohio State University.

A few articles have been written concerning the digestive enzymes present in various flies. Bogdanow (1928) succeeded in breeding eleven continuous generations of *Calliphora erythrocephala* on human excreta and concluded that this fly built up proteins from simple nitrogenous compounds and amino acids. Trypsin was found in the excreta of Calliphorid larvae according to Weinland (1906). Protease, amylase and erepsin in Calliphorid and Lucilia larvae were found by Wollman (1922). Probably the most complete work on digestion of flies was done on *Lucilia sericata* by Hobson (1931), who found traces of amylase in the salivary glands while tryptase, peptidase and lipase were found in the midgut. Proteolytic enzymes were also found in the excreta.

PREPARATION OF MATERIALS FOR ENZYME TESTS.

Full grown *Phormia regina* larvae which had not yet ceased to feed were used throughout the tests. They were starved until their alimentary tracts were empty by placing them in a specially prepared filter that provided for a slow stream of water to pass over them. This procedure, similar to that devised by Hobson, not only kept the larvae moist during starvation, but it also prevented the ingestion of excreta. The alimentary canals of larvae treated in this manner were usually perfectly clean within 6 to 8 hours, and by this method we were certain that any positive tests obtained would be from enzymes located in the tissues of the larvae.

The digestive tracts were then removed from the larvae, after which glycerol extracts were made separately of the salivary glands, crops, anterior-middle- and posterior-portions of the mid-intestines, and hind intestines. The organs, as well as the division of the mid-intestine into three parts, are shown in Figure 1, which illustrates the gross anatomy of *Phormia regina*, having a digestive tract essentially the same as that of *Calliphora erythrocephala* (Lowne, 1890) and *Lucilia sericata* (Hobson, 1931).

When fifty larvae were so dissected each of the various portions was completely macerated in a mortar and stored in vaccine tubes under toluene.

Preparations from the excreta were made by placing one hundred washed larvae in the filter, and allowing 50 cubic centimeters of water to drip over and through them for four hours. This solution was then stored in a similar manner by

placing it in vaccine tubes under toluene. The possibility of regurgitation was not eliminated by this procedure, but this was not essential since we desired to determine all the digestive enzymes emitted by the larvae, as they would all be present in the treatment of a wound.

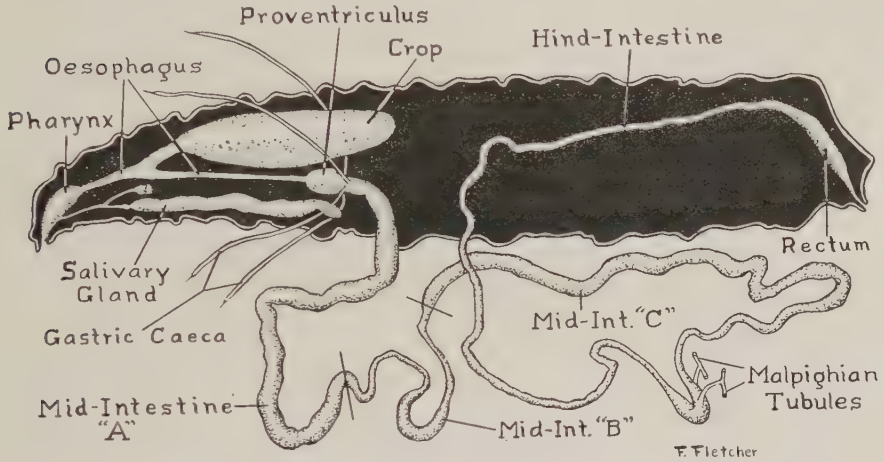

FIG. 1. Gross anatomy of the Blowfly larva, *Phormia regina* Meigen.

DETECTION OF ENZYMES AND HYDROGEN-ION CONCENTRATION.

The methods of enzyme analysis were essentially those devised by Swingle (1928) with certain modifications being made according to Wigglesworth (1927, 1928 and 1929) and Hobson (1931). A very brief summary of the methods used were as follows:

Amylase was tested for by glycerine extracts acting upon .5 cc. of 0.5 per cent starch solution, potassium iodide added after 72 hours incubation at room temperature. The Flückiger test was used to detect the presence of reducing sugars. *Maltase* was tested for by adding glycerine extracts to a 15 per cent solution of maltose, incubated for 72 hours, and the osazone test used to determine the presence of glucose. The test for *Invertase* was the addition of the glycerine extract to 0.5 cc. of 15 per cent solution of sucrose, and, after 72 hours incubation, the presence of fructose and glucose tested by using the osazone test and the presence of reducing sugars by the Fluckiger test. The test for *Lactase* was the same as that for maltase with the exception of using a 3 per cent lactose solution as the

substratum. *Lipase* was tested for by using the Brom Thymol Blue Emulsion test. An emulsion of olive oil and acacia was added to the glycerine extract in which the presence of Brom Thymol Blue and lipase would cause a color change from blue to greenish-yellow after 96 hours incubation. *Trypsin* and *Pepsin* were tested for by using the blood fibrin test as outlined by Swingle, the enzymic action breaking down the fibrin previously stained with Ruthenium Red for the trypsin test, and stained with Amaranth for the pepsin test. *Erepsin* was tested for by using the Modified Sorensen Method as outlined by Swingle.

TABLE I.

Digestive Enzymes in Contaminated Larvae of *Phormia regina* Meigen.

Enzyme	Salivary Glands	Crop	Anterior Mid-gut	Middle Mid-gut	Posterior Mid-gut	Hind Gut	Excreta
Amylase	±	—	—	—	—	—	—
Maltase	—	—	—	—	—	—	—
Invertase	—	—	+	—	—	—	—
Lactase	—	—	—	—	—	—	—
Lipase	—	—	±	—	±	±	±
Trypsin	—	—	+	—	+	+	+
Pepsin	—	—	—	—	—	—	—
Erepsin	—	—	+	—	+	±	±
pH	7.18	7.24	7.15	6.0	7.18	7.54	7.1

The hydrogen-ion concentration was determined in the various portions of the digestive tract as well as the excreta. The fluids from the tracts of freshly-killed larvae were taken up in small capillary tubes, indicators added and the resulting colors compared with a similar set of capillary tubes made up with standard indicators. This method was checked by using a standard micro-comparator set.

The results obtained are indicated in Table I. The plus sign (+) represents a relatively strong reaction, while the negative sign (—) indicates no reaction, and the two signs together (±) point out a weak reaction. These tests were repeated in three different sets of experiments and only once was the presence of *invertase* indicated.

EFFECT OF BACTERIA ON DIGESTION.

A technique was devised for the rearing of aseptic larvae. This was thought advisable for two reasons: first, that it offered a check on controversial results of former investigations, and second, that it provided a means of proving that whatever digestion occurred was due to enzymes present and not to bacteria. Fresh lean beef was ground up and weighed into 12-gram amounts and placed in large test tubes that had been previously autoclaved. This set-up was then heated in a water bath at about 75° C. for 30 minutes on four successive days, thereby killing off the vegetative stages of any bacteria that might have developed after the first and second heatings.

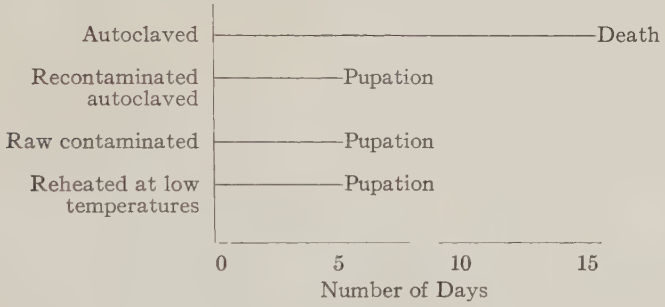

FIG. 2. Rate of development of larvae on varied treatments of the same medium.

After this part of the procedure was finished eggs of *Phormia regina* were implanted that had been previously sterilized by washing them in an alcoholic solution of 1 : 2000 mercuric chloride, about 30 eggs placed in each tube. Check tubes on rate of development were made by placing raw contaminated beef in one tube, recontaminated autoclaved beef in the second, and sterile autoclaved beef in the third. The larvae were allowed to grow without any disturbance of the sterile cotton plugs that closed the test tubes. After the larvae had become full grown, the contents of the test tubes were examined for bacterial contamination by implanting small portions of them in anerobic and aerobic broth tubes, on agar plates, and by stained smears on microscope slides.

The maggots in those tubes that proved to be sterile were then divided into two lots. The larvae of the first lot were allowed to continue their development, and the flies that

emerged were full size and seemed normal in every respect. Some rather significant results were obtained when the rate of development of these larvae were compared with those reared in the check tubes. Figure II illustrates these results graphically, carried only through the feeding period (Haub and Miller, 1932).

At a constant temperature of 29° C., and a relative humidity of 70 per cent, maggots grown in the media made sterile by this low heating method pupated on the fifth day. The same rate of development occurred in those tubes containing raw contaminated beef and recontaminated autoclaved meat. Those maggots grown in the sterile autoclaved meat did not mature at all, but remained for over two weeks in a size corresponding to the second or third instar, and at the end of that time died. This experiment was repeated twice and identical results occurred.

This would seem to indicate that some food factor is destroyed when the meat was autoclaved, that this factor is somehow replaced when this same autoclaved food is contaminated (Glaser, 1924), and that sterilizing at lower temperatures does not destroy this factor at all.

The second lot of maggots, reared by this method was then set aside to be used as a check on the enzymic reactions obtained from the larvae raised on natural contaminated meat as summarized in Table I. The same methods were used for the determination of enzymes as were used previously, except that the maggots were handled under the most aseptic conditions possible. All apparatus used was sterilized. Each maggot was dissected under sterile water, and the instruments used were sterilized in mercuric chloride after each dissection. The portions of the digestive tracts obtained in this manner were then placed in their respective vaccine tubes that had been previously autoclaved, and after the proper chemicals had been added to determine the enzymic reactions, each tube was capped with toluene.

By using these precautions, we were then positive that whatever digestive reactions occurred were due to the presence of enzymes and not to any bacteria that otherwise would have been present.

Hobson (1932a) had previously checked only for the presence of tryptase and pH values in *Lucilia sericata*, and found that tryptase was present in aseptic larvae and that the gut reactions

in them were normal, when he reared the larvae on autoclaved brain mush. Our experiments included the testing for all the enzymes that had been found present or absent in the contaminated larvae, and the aseptic larvae were raised on lean beef. The results obtained are summarized in Table II.

A comparison of the results obtained from those maggots raised in a contaminated medium and those reared aseptically showed that the only difference occurring was a positive test for invertase in the anterior portion of the mid-guts of the contaminated larvae. Furthermore, since the reaction for the

TABLE II.

Digestive Enzymes in Aseptically-reared Larvae of *Phormia regina* Meigen.

Enzyme	Salivary Glands	Crop	Anterior Mid-gut	Middle Mid-gut	Posterior Mid-gut	Hind Gut	Excreta
Amylase	±	—	—	—	—	—	—
Maltase	—	—	—	—	—	—	—
Invertase	—	—	—	—	—	—	—
Lactase	—	—	—	—	—	—	—
Lipase	—	—	±	—	±	±	±
Trypsin	—	—	+	—	+	+	+
Pepsin	—	—	—	—	—	—	—
Erepsin	—	—	+	—	+	±	±
pH	7.0	7.0	6.9	6.6	6.9	7.2	6.8

presence of invertase occurred only once in the three times that the experiment was repeated, it suggests that the hydrolysis of sucrose was not caused by the enzyme but probably by bacteria.

SUMMARY.

In the treatment of osteomyelitis, the role of digestion in blowfly larvae is a significant one, being interrelated with the success of the treatment. A study was made to determine enzymes present, the effect of bacteria on digestion, and the analysis of the excreta.

The only enzymes found present in various portions of the digestive tract were amylase, lipase, trypsin and erepsin.

A method was devised to raise flies from egg to adult aseptically by repeated heatings of the media at low temperatures. It was demonstrated that some food factor is destroyed when the media was autoclaved, that this factor is somehow replaced when this same autoclaved media is recontaminated, and that sterilizing at lower temperatures does not destroy this factor at all.

By this method a check was provided on enzymic determinations in contaminated larvae, and the only significant difference obtained was in the test for invertase; it was found present in the contaminated larvae but not in the larvae reared aseptically, which would seem to indicate that the hydrolysis of sucrose was not caused by the enzyme but probably by bacteria.

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Man and Metals.

This is the story of man's advances as viewed from the standpoint of mining and metals. From the "Age without Metals" to our modern times the author has given us an insight into the various aspects of mining and metals, and how they have tended to shape our civilization. He considers the use of metals and manners of obtaining them from earliest times through the Mediterranean civilizations, the Medieval developments of the Old World, then in the New World starting with the Conquest of Peru and coming down to the mining in Australia, and finally to Cecil Rhodes and Southern Africa. Coal and iron are considered as separate from the rest.

This impressive work should not only be of interest to the mining engineer, the metallurgist, and the geologist, in whose special field it lies; but also to the economist, the student of civilization and development of culture, and to all who are interested in the history of our development.

The work is well written and very readable, sustaining interest throughout its many pages. The author is to be congratulated on the book, the entire make-up of which is pleasing.—W. Berry.

Man and Metals, by T. A. Rickard. Two volumes, 1061 pp., 108 ill. New York, Whittlesey House, (McGraw-Hill Book Co.), 1932. $10.00.

Earth History.

This volume on geology is the first of the Century Earth Science Series and handles Earth History in an entirely new manner. There are four main sections; in the first are considered various physical processes, the time scale and cycles of earth history. The second takes up the physical history of the continents. The third considers the biologic history, and the last, "Man and Earth History."

The arrangement and manner of presentation are new and unusual in what might be called introductory geology. The style is good and the text is not burdened with technical terms or involved explanations. For the general reader it is far ahead of the general run of introductory geologies, but I question its use as a text in introductory geology, as it does not seem to be exactly "teachable." As supplementary reading or general reference it is excellent.

The book is well gotten up and is well handled by the publishers. It should find a ready place in general reading and as a supplementary text.

Willard Berry.

Earth History, by L. C. Snyder. 675 pp. New York, The Century Co., 1932.

THE EFFECT OF HIGH CONCENTRATIONS OF DISSOLVED OXYGEN ON SEVERAL SPECIES OF POND FISHES.

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INTRODUCTION.

The investigation of this problem was undertaken for two reasons: first, because investigations of the oxygen content of pond waters have shown that at times when photosynthesis is proceeding at a rapid rate, the water is supersaturated with dissolved oxygen and second, because of the coming into use of compressed oxygen in aerating fish during transportation. In a paper by Wiebe (1931) it has been shown that in one of the fish ponds the water was saturated 189.50%. Unpublished data obtained at this laboratory by Mr. A. M. MacGavock during the summer of 1931 show that in one of the bass ponds the percentage of saturation of the surface water ranged from 164% at 8:00 A. M. to 304% at 5:00 P. M. The next morning at 7:00 o'clock it was down to 153%. The mass production of O_2 in this case was due to an enormous growth of the blue-green alga, *Anabaena*. In 1928 the water in one of the ponds contained 4.68 p. p. m. of dissolved oxygen at 8:00 A. M. and 15.90 p. p. m. at 3:00 P. M. of the same day, Wiebe (1930).

The effect of high concentration of oxygen on fishes has been studied by Shelford and Allee (1913), by Wells (1913), by Haempel (1928), and by Plehn (Praktikum der Fischkrankheiten). Hubbs (1930) has investigated the effect of nascent oxygen on fish.

METHOD OF PROCEDURE.

The containers used in these experiments were large glass carboys, an iron preserving kettle, and specially constructed fish cans. These containers were so equipped that they could be attached to a tank of compressed oxygen and with an outlet so that water could be displaced by oxygen and samples withdrawn without opening the container. In the glass carboy it was possible to observe the reaction of the fish during the

experiment. This was not possible in the other containers mentioned. The ability of the fish to survive the experiments for shorter or longer periods was used as a criterion.

The experiments are primarily of two types and the results are presented under two sections, A and B. In the first group of experiments the fish were subjected to high concentrations of oxygen under atmospheric pressure with an atmosphere of pure oxygen over the water. In the second group the fish were subjected to pressure of varying degrees. In most of these pressure experiments a layer of pure oxygen was maintained over the surface of the water. The pressure readings were taken in whole and in half pound intervals on a pressure gauge. Then the gauges were calibrated for each scale interval against a mercury monometer. More specific details are given below.

Species of fish used were: largemouth black bass, *Micropterus salmoides*, (Lacepede); smallmouth black bass, *M. dolomieu* (Lacapede); the black crappie, *Pomoxis Sparoides* (Lacepede); the white crappie, *P. annularium* (Rafinesque), the bluegill, *Lepomus incisor* (C. and V.); the golden shiner, *Notemigonus crysoleucas* (Mitchill); the common goldfish, *Carassius auratus* (L.); the orange spotted sunfish, *Lepomus humilus* (Girard); the brook trout, *Salvelinus frontinalis* (Mitchill), and the rainbow trout, *Salmo shasta* (Jordan).

Section A.

Experiments to show, (1) that various species of pond fish can live in water with a stratum of pure oxygen over the water, and (2) that they can stand a sudden transition from water with a low oxygen content to water of a relatively high oxygen content and the reverse.

The experiments were carried out in large glass carboys. The procedure was as follows: The container was completely filled with water before the fish were introduced. After the fish had been put in the container the latter was stoppered tightly and connected to a tank of compressed oxygen. The gas was released close to the bottom of the container. The carboy was also equipped with an exit tube so that some of the water could be displaced by oxygen. In this manner a layer of pure oxygen was established. Preliminary experiments have shown that if the oxygen is introduced near the bottom of the container, it takes only a relatively short time until the

concentration of oxygen becomes fairly uniform throughout the entire mass of water. Samples for oxygen determination were taken close to the bottom. In some experiments, No. 2, for instance, samples were taken at different depths after the fish had been introduced, but no significant difference in the amount of oxygen was discovered. In some instances the oxygen in the experimental container was increased by bubbling oxygen through the water before the fish were introduced. This was done in order that it might be determined if fish can stand a sudden transfer from low to high oxygen tension.

Experiment 1.

Smallmouth Black Bass Fry.

In this experiment 160 smallmouth bass fry ranging in size from 0.5″ to 0.75″ were used. They were taken from pond water having an O_2 content of 10.62 p. p. m., a pH of 9.3, and a temperature of 23.5° C.

Table I.

Shows O_2 in Parts per Million, Temperature in Degrees Centigrade, and pH Value for Experiment Number 1.

Date	Hours	O_2	pH	Temperature	Remarks
5–31–31	2:30 P. M.	20.75	7.3	23.5	Initial condition in carboy when fry were introduced.
5–31–31	5:00 P. M.	28.38	.....	.....	.....
5–31–31	9:45 P. M.	30.71	.....	.....	.....
6– 4–31	5:30 A. M.	30.71	.....	.....	When the experiment was terminated the CO_2 amounted to 25 p. p. m.
6– 4–31	3:00 P. M.	30.05	7.25	19.5	.....

and placed in a carboy whose water had an O_2 content of 20.75 p. p. m., a pH of 7.3, and a temperature of 20.0° C. After the fish had been put in the container the latter was stoppered tightly and some of the water displaced by oxygen, thus creating a layer of pure oxygen over the surface of the water. The experiment was begun at 2:30 P. M., May 31, and was terminated at 3:00 P. M., June 1st. Sufficient fresh water was added at this time to again fill the carboy completely reducing the O_2 to 21.58 p. p. m. Additional data for O_2, etc., are shown in Table I.

No fish were lost during this 24-hour experiment. When the carboy was opened and some water fleas and small shiners put in, the bass fed on them very readily. The fish were kept under

observation in this carboy for a week, the water being aerated with compressed air. No fry died during this period.

At no time did these fish show any signs of uneasiness while they were under an atmosphere of pure oxygen. This together with the fact that they survived so well and took food so readily would seem to show that this treatment had no deleterious effect on these little fish.

Experiment 2.

Largemouth Black Bass Fry.

The procedure in this experiment was the same as in number one. The fish used were 150 largemouth fry 0.5″ to 0.75″ in length. They were transferred from water having a pH of 7.9, O_2 content of 7.21 p. p. m., and a temperature of 24.0° C., to water having a pH of 7.5, an O_2 content of 27.25 p. p. m., and a temperature of 20.0° C. This experiment was begun at 10:00 A. M., June 4.

At 1:30 P. M. two oxygen samples were taken from this carboy: one at 2″ below the surface and another at 8″. The former sample had an O_2 content of 31.99 p. p. m., and the latter had 31.44 p. p. m. At 5:20 P. M. two samples were taken at 1.5″ and 7.5″ respectively. The O_2 content of both samples was 32.75 p. p. m.

At 5:00 P. M. the O_2 1″ below the surface was 30.65 p. p. m., and at 7″ below the surface it was 29.87 p. p. m. At 9:00 A. M. June 6, the O_2 7″ below the surface was 32.75 p. p. m., pH 7.1, free CO_2 15.1 p. p. m., and temperature 19.0° C. The experiment was discontinued at this time. The carboy was refilled with water and the latter aerated with compressed air. The fish were kept under observation until June 11. Up to that time three fry had died.

It would appear from the above that the sudden transfer from an O_2 content of 7.1 p. p. m. to 27.25 p. p. m. did not harm these bass fry and that they could live in water under an atmosphere of pure oxygen.

Experiment 3.

Smallmouth Black Bass Fingerlings.

Procedure same as in number two. Fish used were 75 smallmouth bass having an average length of 1.75″. They were transferred from water with an O_2 content of 6.42 p. p. m. to water whose O_2 content was 22.7 p. p. m. The transfer was made at 11:00 A. M. July 10. The temperature in the trough was 21.5° C. and the pH 7.6; the temperature in the carboy was 23.0° C. and the pH 7.2. For additional data on O_2, etc., see Table II.

The experiment was discontinued at 9:00 A. M. July 12, and the fish transferred to water having an O_2 content of 6.0 p. p. m. The fish seemed none the worse for this experiment.

They learned to take artificial food in a few days and acted normally in all respects. No fish were lost during the experiment. The same was true of the control fish. These fish were kept under observation until July 27, i. e., until 17 days after the experiment was begun. During this period only one experimental bass was lost—this one was dead on July 13. The control fish all survived up to this time.

TABLE II.

Shows O_2 and Free CO_2 in Parts per Million, Temperature in Degrees Centigrade, and pH Value for Experiment Number 3.

Date	Hour	O_2	pH	Temperature	Free CO_2	Remarks
7–10–31	11:00 A. M.	22.7	7.2	23.0	.....	Initial conditions
7–10–31	1:00 P. M.	24.69	.....	.....	.....	
7–11–31	8:00 A. M.	20.28	7.0	24.0	35.2	
7–11–31	5:00 P. M.	21.2	7.0	23.5	39.6	
7–12–31	8:00 A. M.	18.2	6.9	23.0	47.5	

The above results would certainly seem to indicate that this size of smallmouth bass could live in water with a superstratum of pure oxygen for a period of 46 hours. Moreover, that they could stand a sudden transition from water having an O_2 content of 6.42 p. p. m. to water having 22.7 p. p. m., and from 18.2 p. p. m. to 6 p. p. m.

EXPERIMENT 4.

Largemouth Black Bass Fingerlings.

This is an exact repetition of number three, only that 50 largemouth bass having an average length of 2.64″ were used instead of smallmouth bass. This experiment was carried on simultaneously with number three and the values for O_2, pH, free CO_2, and temperature are essentially the same as in number three, hence they are omitted. Only the results as far as the fish are concerned are recorded here.

Three bass were lost during this experiment while none of the controls died during the 44-hour period. The remaining fish were kept under observation until July 27. Up to that time 4 more of the experimental bass had died and two of the controls. Although the losses here are greater than in number three, it is still very doubtful that the death of these fish was caused by the high oxygen. The fact that two of the controls also died points the other way. Also the experimental fish were handled more than the controls. This may have had something to do with the greater mortality.

Experiment 5.

Trout Fingerlings.

The procedure in this experiment is the same as in the preceding experiments. The fish used were 10 rainbow and 10 brook trout that had an average length of about 2.5″ to 3″. The fish were taken from water having an O_2 content of 5.68 p. p. m., and 6.68 p. p. m. They were transferred to water having an O_2 content of 17.1 p. p. m., free CO_2 1.5 p. p. m. The temperature varied from 22.0 to 23° C. during this experiment. Additional observations made during the test period which lasted from 8:00 A. M. July 13 to 8:30 A. M. July 15, are shown in Table III. At this time enough fresh water was added to reduce the O_2 to 10.9 p. p. m. The fish were all in good condition. They were kept under observation until July 23. Only one trout had died up to that time. Hence it seems that to live for 48.5 hours in water under a super-stratum of oxygen produced no ill effect in these fish. (It may be noted here that the temperatures that prevailed during this experiment were unfavorable for these small trout.)

Table III.

Shows O_2 in Parts per Million, Temperature in Degrees Centigrade, for Experiment Number 5.

Date	Hour	O_2	Temperature	Free CO_2
7–13–31	4:30 P. M.	23.7	22.0	.....
7–14–31	8:30 P. M.	26.2	22.5	.....
7–15–31	8:30 P. M.	37.6	22.5	.....

From the results of this experiment it would appear that these small trout could stand a sudden and extensive change in the concentration of dissolved oxygen.

Experiment 6.

Black Crappie and Bluegill Fingerlings.

The experiment was begun at 11:00 A. M. May 23. At that time 12 black crappie 2.5″ to 4″ and 23 bluegill 1.5″ to 2.5″ were transferred to an experimental carboy, and 13 black crappie and 10 bluegill of the same size to a control carboy. In the control the water was aerated with compressed air. In the experimental carboy the O_2 content of the water had been raised by bubbling O_2 through the water before the fish were introduced. After the fish were in the carboy some of the water was displaced with O_2. At the time of the transfer the O_2 in the retaining trough was 3.32 p. p. m., in the experimental carboy the O_2 amounted to 22.41 p. p. m., and in the control 5.48 p. p. m. During the test period the O_2 in the control varied between 4.15 p. p. m. and 7.8 p. p. m. The temperature in both carboys ranged from 17.0° C. to 17.5° C.

Additional oxygen values for the experimental carboy are shown in Table IV. At the end of the experiment the free CO_2 amounted to 7.5 p. p. m. in the experimental carboy. The experiment was terminated at 1:00 P. M. May 25.

At the end of the experiment one of the experimental bluegill was sick and two of the control crappie were dead. Up to May 30, 21.7% of the experimental and 40.0% of the control bluegill had died. At the same time 8.33% of the experimental crappie and 46.1% of the control crappie had died. After that no losses occurred for several days, and the fish were liberated. From these results it would appear that

TABLE IV.

Shows Values for O_2 in Parts per Million for Experiment Number 6.

Date	Hour	O_2	Date	Hour	O_2
5–23–31	1:00 P. M.	25.89	5–24–31	10:00 A. M.	24.88
5–23–31	6:00 P. M.	27.89	5–25–31	8:00 A. M.	21.08
5–23–31	9:45 P. M.	26.72	5–25–31	1:00 P. M.	20.92

these fish had been benefited by the high concentration of oxygen. (It may be well to mention here that these were rescued fish.) The fish that survived the experiment took food readily.

EXPERIMENT 7.

Two-year Old Bluegill.

The procedure in this experiment was the same as in number 5, and it was begun at 4:00 P. M. May 26. In this experiment 10 two-year old bluegill were used. The same number was used for a control. The fish were taken from water having an O_2 content of 2.65 p. p. m. The water in the experimental carboy had an O_2 content of 25.73 p. p. m. at the time the fish were introduced. During the test period the O_2 ranged from 24.03 p. p. m. to 31.87 p. p. m., while the range in the control was from 4.48 p. p. m. to 8.13 p. p. m. The temperature ranged from 18 to 21.5° C. The experiment lasted for 45 hours. At the end of the experiment the fish were transferred to separate compartments of an aquarium. The O_2 in this aquarium amounted to 6.31 p. p. m. Each carboy had one dead fish and one sick fish at the end of the experiment. The dead as well as the sick fish showed definite skin lesions. (The two-year old bluegill had been given a copper sulphate bath a few days before the experiment was begun. They were badly fungused before the treatment.) Two of the experimental and one of

the control fish were dead on May 29; all had definite skin lesions. The fish were kept under observation for two more weeks, but no more losses occurred. The fish were now entirely free from fungus and took food very readily.

Total loss of fish was three experimentals and two controls. Hence it may be concluded that the high oxygen and the atmosphere of pure O_2 over the water had no ill effect on these fish.

Experiment 8.

Largemouth Bass Fingerlings.

Procedure was as in experiment number 7. Fish used were: 6 largemouth bass from 4.25″ to 5.25″ in length. The O_2 content of the water in the experimental carboy had been raised to 40.33 p. p. m. before the fish were introduced. The fish were transferred to this carboy from water having an O_2 content of 5.67 p. p. m. Observations on O_2, and temperature are shown in Table V.

Table V.

Shows O_2 in Parts per Million, and Temperature in Degrees Centigrade for Experiment Number 8. The Per Cent Saturation is Referable to an Atmosphere of Ordinary Air.

Date	Hour	O_2	Temperature	% Saturation
1–27–31	10:10 A. M.	40.33	16.5	410
1–27–31	1:00 P. M.	37.49	16.5	381
1–27–31	4:00 P. M.	35.97	16.5	366
1–28–31	9:00 A. M.	29.43	17.0	302

These fish showed no signs of restlessness when put in the water of high oxygen content. They all survived the experiment and were kept under observation for several days before they were returned to the retaining trough. The sudden transfer from low to high oxygen and the 23-hour stay in water under an atmosphere of pure oxygen had no deleterious effect.

Additional experiments involving more than a hundred largemouth bass, several smallmouth bass, more than 200 golden shiners, several bluegill, at least 50 goldfish, and several crappie all gave essentially the same results.

Section B.

This section deals with the results of experiments designed for the purpose of determining the ability of fish to live under small pressures.

EXPERIMENT 9.

Bass, Sunfish, Goldfish and Shiners.

In this experiment an iron preserving kettle was used as a container for the fish. The procedure was essentially the same as in Section A except that no water was displaced and that the oxygen was introduced over the surface of the water instead of being bubbled through the water from the bottom. The result of this modified procedure was a much lower O_2 content of the water, namely, 13.64 p. p. m. at the conclusion of the experiment. The temperature ranged between 17.5 and 18.0° C.

The fish used in this experiment were: 4 largemouth black bass (4.25″ to 5.5″), 2 goldfish (3″ to 3.5″), 1 green sunfish (2.25″), and 8 golden shiners (4″ to 5.5″; this does not include two smaller shiners eaten by the bass in the course of the experiment).

In this experiment a pressure of 7 to 9 lbs. was maintained from 11:30 A. M. to 1:40 P. M. Then from 1:40 P. M. to 11:30 P. M. a pressure from 12 to 13.2 lbs. was maintained. The fish were taken out at 12 o'clock midnight and transferred to water that had an O_2 content of 13.62 p. p. m. No fish except the two shiners consumed by the bass were lost in the experiment. However, one of the larger shiners had several blood stains on its sides. The rest of the fish showed no visible signs of injury. The apparently injured shiner acted perfectly normal, and when these fish were returned to the holding troughs after having been kept under observation for two weeks, was alive and healthy. Since no fish were lost and since the fish acted normally and took food readily, it is concluded that an increase in pressure of from 0.46 to 0.88 atmosphere above normal exerted no deleterious effect on these fish.

EXPERIMENT 10.

Bass, Goldfish and Shiners.

This experiment was carried out in two stages. The pressure cooker was again used as a container. In the first part of the experiment the O_2 was bubbled through the water while in the second part it was introduced over the surface of the water. The fish used in part 1 were: 3 largemouth bass (4″ to 5″), 1 smallmouth bass (5″), 3 goldfish (3″ to 3.25″), and 2 shiners (3″ to 3.25″—this does not include several small shiners eaten by the bass in the course of the experiment). The pressure

records for part 1 are shown in Table VI. The initial O_2 was 7.92 p. p. m. at a temperature of 17.5° C. At the conclusion of part 1 the O_2 was 38.5 p. p. m. at a temperature of 16.5° C.

TABLE VI.

Shows Pressure Reading for Part One of Experiment 10 in Pounds.*

Hour	Pressure	Hour	Pressure	Hour	Pressure
10:35 A. M.	0 lbs.	11:20 A. M.	18.6 lbs.	12:40 P. M.	17.6 lbs.
10:55 A. M.	12.1 lbs.	11:25 A. M.	17.1 lbs.	1:05 P. M.	17.6 lbs.
11:00 A. M.	12.1 lbs.	11:30 A. M.	17.6 lbs.	1:10 P. M.	17.6 lbs.
11:04 A. M.	15.4 lbs.	11:35 A. M.	18.6 lbs.	1:15 P. M.	17.6 lbs.
11:05 A. M.	17.6 lbs.	11:40 A. M.	19.6 lbs.	1:20 P. M.	7.85 lbs.
11:10 A. M.	17.6 lbs.	11:45 A. M.	17.6 lbs.	1:25 P. M.	4.30 lbs.
11:15 A. M.	17.6 lbs.	12:05 P. M.	17.6 lbs.	1:30 P. M.	0 lbs.

Part 2 of this experiment was begun at 2:15 P. M. after a 3-inch bluegill had been added. The rest of the fish were the same as in Part 1. The pressure was raised again, but instead of bubbling the O_2 through the water, it was introduced over the surface of the water. The result was that the dissolved oxygen increased only very little during Part 2 of this experiment, namely, from 38.5 p. p. m. to 39.82 p. p. m. The pressure records for Part 2 are given in Table VII.

TABLE VII.

Pressure in Pounds for Part Two of Experiment 10.

Hour	Pressure	Hour	Pressure	Hour	Pressure
2:15 P. M.	0 lbs.	2:45 P. M.	18.6 lbs.	3:45 P. M.	17.6 lbs.
2:17 P. M.	7.17 lbs.	2:55 P. M.	18.6 lbs.	4:00 P. M.	17.6 lbs.
2:30 P. M.	7.85 lbs.	3:05 P. M.	18.6 lbs.	4:20 P. M.	17.6 lbs.
2:40 P. M.	12.10 lbs.	3:20 P. M.	18.6 lbs.	4:40 P. M.	17.6 lbs.

During the interval from 4:40 P. M. to 4:50 P. M. the pressure was reduced from 17.6 lbs. to 0 lbs. The cover was then removed and the fish examined. The fish were on the bottom of the container apparently perfectly at ease.

The fish were left in this container until 8:00 A. M. the next morning, when they were transferred to a holding trough. The fish were all alive and active. Several small shiners had

*_Note._—During the intervals between readings the pressure was maintained between the values shown for consecutive readings, i. e., during the interval between 11:20 A. M. and 11:25 A. M. the pressure was never below 17.1 lbs. or above 18.6 lbs.

been eaten by the bass during the experiment. The smallmouth bass had a shiner in its mouth at the time of the transfer. These fish showed no sign of injury whatsoever.

This experiment suggests that these fish could adapt themselves rapidly to an increase in pressure slightly in excess of 2 atmospheres.

In the last two experiments due to the peculiar construction of the iron kettle it was not possible to create a layer of pure oxygen over the water.

Experiment 11.

Bass, Goldfish, Sunfish and Golden Shiners.

In this experiment fish were subjected to a pressure of 9.85 lbs. for 10.5 hours with a layer of pure oxygen over the surface of the water. A glass carboy was used as a container for the 5 largemouth (3.5″ to 5.5″), 3 goldfish (3″ to 3.5″), 2 bluegill (2.75″ to 3″), and 15 golden shiners (2.5″ to 5″). For a few minutes during and after the pressure was raised the fish were slightly restless, but they soon became quiet and remained so during the entire test period. (This does not mean motionless, for they moved around freely at all times.)

This experiment was begun at 11:00 A. M. February 13. The following values for O_2 obtained during this experiment at the time indicated: 12:15 P. M., 19.53 p. p. m.; 2:40 P. M., 26.91 p. p. m.; 7:45 P. M., 39.06 p. p. m.; at 9:30 P. M., 42.53 p. p. m. At this time the oxygen tank was disconnected and the pressure allowed to go down to zero in a few minutes. The fish were kept in the carboy until 8:00 A. M. of the next day, at which time they were transferred to an aquarium. The oxygen in the carboy still amounted to 26.26 p. p. m. at the time of transfer. The fish were all in fine condition and apparently had suffered no adverse effect from the conditions of the experiment. On February 28, i. e., fourteen days after the experiment was terminated, all the fish with the exception of the two smallmouth were still alive and healthy. The two smallmouth were dead on February 17, three days after the termination of the experiment. At the time of their death the O_2 in the aquarium was down to 1.08 p. p. m. This was apparently too low for the smallmouth. The other species could survive the low O_2 because they are less timid and come and snap for air at the surface more freely.

Note: Some of the bass used in this experiment were still alive in June and July, when they were used in experiments to determine the amount of arsenic in bass.

Experiment 12.

Bass and Trout.

The object of this experiment was to determine if fish considerably smaller than the ones used in experiments 9 to 11 could live under pressure with a super-stratum of pure oxygen. The container used was built of galvanized iron and was air tight. It was also equipped with an inlet for the gas and an outlet for water. The procedure was as follows: The can was completely filled with water before the fish were introduced. After the fish had been put in and the cover tightened down, the can was connected to an oxygen tank and some of the water displaced so that a layer of pure oxygen was formed over the surface of the water. After sufficient water had been displaced the pressure was raised 13.23 lbs. This pressure was attained at 2:30 P. M. July 14, and maintained until 8:30 P. M. of the same day. During the intervals between 8:30 P. M. and 7:30 A. M. the next morning the pressure went down to 11.15 lbs. At 7:30 A. M. the pressure was again raised to 13.23 lbs. by the introduction of additional O_2 and maintained there until 2:45 P. M. July 15. At this time the pressure was reduced to zero rapidly and the fish removed from the water in the can that had an O_2 content of 41.0 p. p. m. to water having an O_2 content of 7.3 p. p. m. The temperature ranged from 21.5° C. to 22.5° C. during the course of this experiment.

Table VIII.

Record of Fish.

Species	Size of Fish	Dead at End of Experiment	Alive at End of Experiment
Largemouth bass	1.25″–2″	3	33
Smallmouth bass	1″ –1.4″	0	21
Trout.....	2.5″	2+1 sick	7
Total dead....	.....	5+1 sick	Alive 61

These results show that these small fish could stand a pressure of from 11.14 to 13.23 lbs. in excess of one atmosphere for a period of 24 hours. The large-mouth bass had been seined in one of our ponds just before the experiment was begun. While this experiment was in progress four of the largemouth in the retaining trough died, as did also several trout. It must be stated here that the temperatures prevailing during the experiment were beyond the range that is normal for these species of trout (rainbow and brook). From the time the experiment was terminated (July 15th) until August 11, only one individual of each species of bass had died—one large-

mouth was dead on July 30 and one smallmouth on August 6. The trout died as follows: July 16, 1; July 19, 1; July 26, 1; July 28, 3. The remaining trout has disappeared, probably eaten by the bass. All the trout in the retaining pond had likewise died. Hence the death of the trout may not be attributed to the fact that these fish were kept under pressure for 24 hours. Experiment 5, Section A, proves that it was not the high O_2 that proved detrimental.

Experiment 13.

Bass, Goldfish and Sunfish.

Procedure here was the same as in number 12. Fish used were 9 largemouth bass (4″ to 5″), 9 goldfish (2.5″ to 3.5″) and 12 bluegill (2.0″ to 3.0″). These were all tame fish and accustomed to taking artificial food. This experiment was begun at 4:30 P. M. July 14.

A pressure of 13.61 lbs. was attained at 4:45 P. M. At 8:30 P. M. the pressure was down 12.99 lbs. At 7:30 the next morning the pressure was down to 9.85 lbs. The pressure was raised to 12.99 lbs. at that time and retained there until 4:45 P. M. At 2:45 P. M. the O_2 amounted to 41.2 p. p. m. The temperature varied around 22° to 23° C. during this experiment. The fish were taken out of the experimental container at 4:45 P. M. and put in water having an O_2 content of 7.3 p. p. m. When the fish were taken out 3 bluegill were dead. The rest of the fish were apparently in good condition; they immediately began to take minnows and artificial food. On July 16 another bluegill was dead. No additional losses had occurred up to September 8. Apparently then most of these fish were not affected adversely by a pressure ranging from 9.85 to 13.61 lbs. in excess of one atmosphere for 24 hours.

Experiment 14.

Bluegill.

Procedure as in 12 and 13. Fish used were: 30 bluegill from 2″ to 3″ in length. (These fish had recently been rescued from a slough in Illinois.) The fish were placed in the container at 9:45 A. M. July 16 and the pressure raised promptly to 13.23 lbs. This pressure was maintained for 24 hours by keeping the oxygen tank connected with the fish container. Just before the experiment was ended at 9:45 A. M. July 17, the O_2 in the container was 39.2 p. p. m. The temperature ranged from 21° to 22° C. during the course of the experiment. Thirty minutes were used this time to reduce the pressure to zero. When the can was opened, two fish still alive were floating on the surface of the water. The other fish were all at rest on the bottom. All of these fish were then transferred to water having an O_2 content of 10.6 p. p. m. The two sick fish recovered to some extent after the transfer so that at 4:00 P. M. all the fish were swimming around naturally. On July 18, one bluegill was dead, and on the 19th 3 more were dead. Two of these showed skin lesions. The rest of the fish were feeding at that time.

Several bluegill in the retaining trough which served as controls had also died during this time. From July 19 to September 8 no additional fish had died. In view of the fact that two of these dead fish showed definite skin lesions and also since some of the control fish died, the assumption seems warranted that the death of these fish was not caused by the pressure and that healthy bluegill of this size could exist under a pressure of 12.23 lbs. under a super-stratum of pure oxygen for 24 hours. The transfer from water having an O_2 content of 39.2 p. p. m. to water having 10.6 p. p. m. was also withstood by these fish.

Experiment 15.

Crappie and Sunfish.

The procedure in this experiment is the same as in the preceding one. Fish used were: 7 crappie ranging in size from 4″ to 5½″, 3 bluegill (3.25″ to 4.75″), and 1 orange spotted sunfish (2.75″). A pressure of 12.99 lbs. was attained at 1:00 P. M. July 29. At 4:00 P. M. the pressure was still 12.99 lbs., but at 9:00 P. M. it was up to 13.45 lbs. At 7:30 the next morning the pressure was down to 12.4 lbs. This pressure was maintained until 1:00 P. M., when it was reduced to zero fairly rapidly. Before the pressure was released the O_2 amounted to 38.2 p. p. m. When the can was opened, 3 crappie were on their sides but still alive. The fish were taken out at 1:20 P. M. and transferred to water having an O_2 content of 7.1 p. p. m. Two of the sick crappie recovered in a few minutes, but the third one did not recover fully and on August 1 it was dead. The injury to the crappie was probably due to too rapid depression. The crappie that died had an injured air bladder. Four of the crappie, the bluegills, and the orange spotted sunfish showed no ill effects at any time. Up until October 1 only the one crappie had been lost, i. e., one fish out of eleven. Hence it would appear that a pressure of from 12.99 to 13.45 lbs. exerted for 24 hours is not harmful to these larger bluegill and crappie.

Discussion of Results.

The results given above seem to show that the species of fish used in these experiments can for short periods of time endure relatively high concentrations of dissolved oxygen without producing any immediate ill effects. From the data of some of the experiments it would appear that apparently no ill effects had been produced considering the length of time that the fish were kept under observation. (See especially Experiments 3, 4, 13, 14 and 15.) It is also quite apparent that pressures as high as 13 lbs. can be endured for 24 hours. And pressures as high as 15 to 19 lbs. for a couple of hours. It has not yet been determined whether these fish could endure these higher pressures for longer periods of time.

The phenomenon of air bubbles that was observed by Shelford and Alee (1913) was absent entirely in these experi-

ments. These authors, under the heading "Effect of a great excess of nitrogen and oxygen," record the occurrence of gas bubbles developed in the fins of several species of fish. The water they used contained excess gas to the extent of 1 c. c. per litre of both nitrogen and oxygen. In my experiment the water was supersaturated with oxygen to a very much higher degree. The per cent of saturation has been recorded only in one of our experiments (Number 8), there it reached 410%, i. e., 310 per cent supersaturation. It may, therefore, be assumed that the gas bubbles referred to by these authors were due to the excess nitrogen and not the slight excess of oxygen.

Plehn attributes the occurrence of exophthalmus in trout to the supersaturation of the blood with oxygen. She says, moreover, "dass auch Cypriniden Barsche (Barsche-yellow perch as well as our two species of black bass) wahrscheinlieh alle Fische—durch zuviel Sauerstoff zugrunde gehen. Bei ihnen ist Gasblasenbildung aeusserlich nicht sichtbar—Als auffallendstes Merkmal wird nicht selten eine Truebung der Hornhaut und bei laengerer Dauer auch eine linsen truebung beobachtet." No cases of exophthalmus, opaque lense, or darkening of the cornea were observed in the course of these experiments. She also speaks of the phenomena of irritability and convulsions. These effects were noticed in some of the experiments where larger numbers of the large bass fingerlings were used. In one experiment the details of which have not been included in the text, the bass began to stampede when disturbed. (This sometimes occurs under normal environmental conditions where the fish are confined.) This was on the third day after they had been in a container in which the oxygen was raised suddenly from 5.72 p. p. m. to 20.90 p. p. m., and then permitted to go down gradually. On the third day the O_2 amounted only to 10.41 p. p. m., but the CO_2 amounted to 150 p. p. m., and it was probably the latter that caused the nervousness in the fish. The fish all recovered when transferred to water of a lower O_2 and CO_2 content. In another experiment the fish began to show signs of discomfort on the fourth day of the experiment when the O_2 was down to 6.73 p. p. m. (Temperature, 17.0° C.) The CO_2 was in excess of 200 p. p. m. and was undoubtedly the factor responsible for the great irritability. The assumption that the irritability was not produced by the excess oxygen present at the beginning of the experiment is borne out by the fact that in several

experiments where even higher concentrations of O_2 were used no such irritability was developed.

Haempel (1928) exposed trout (rainbow and brook), carp, (*Cyprinus carpio* L.), Schill (*Lucioperca sandra* Cuv.) and Schleie (*Tinca vulgaris* Cuv.) to high concentrations of oxygen. The highest concentration of O_2 used by him on the trout (adults) was 45.18 p. p. m., the highest used on the carp (adults) 42.61 p. p. m. He noticed one case of opaque lens, but no signs of exophthalmus and of gas bubbles. He gives the results roughly as follows: A marked restlessness, which in the case of the Salmonidae ended in a stampede, marked by an increase in respiration (dyspnae). The young fishes (specially Salmonidae) are seriously injured. They soon turn on their back and pass into a condition of paralysis which he has designated as an "O_2—Narkose." If the exposure to high O_2 is continued the fish die. According to Haempel, the young Salmonidae are more sensitive to the excess of O_2 than the adults. Loosanoff (unpublished data) found that the adult salmon were more sensitive to an excess of oxygen than the fingerlings were. Haempel also calls attention to an excessive production of slime. This was not observed in the experiment reported in this paper. Difference in the rates of respiration were observed.

The change in the rate of respiration for two bass and two bluegill were determined. The results showed that there is a pronounced tendency towards a reduction in the rate of respiration as the concentration of oxygen is increased.

SUMMARY.

(1) Several species of fish have been subjected to higher concentrations of dissolved oxygen when an atmosphere of pure oxygen was maintained over the surface of the water and also with a super-stratum of pure oxygen under pressure.

(2) Several species of fish have been subjected to sudden transfers from low O_2 to high O_2 and the reverse.

(3) The results show (a) that some species of fish tolerate large and sudden changes in the concentration of O_2 in either direction, namely, from 5.67 p. p. m. to 40.33 p. p. m. (Experiment 8), and from 41.2 p. p. m. to 7.3 p. p. m. (Experiment 13); (b) that these fish can live in water containing a large excess of dissolved oxygen with a super-stratum of pure oxygen over the surface (Experiment 9); (c) that several species of fish can stand

a pressure of 10 to 13 lbs. for a period of 24 hours (Experiments 12–15) and pressures from 15 to 19 lbs. for shorter periods (Experiment 10). Longer periods not investigated.

(4) The increase in dissolved oxygen is followed by a slowing down of the respiratory movements.

(5) No instances of exophthalmus, opaqueness of the lens, and of the formation of gas bubbles were observed.

(6) No fish were observed to lose their equilibrium except in a few instances in the pressure experiment where depression occurred too rapidly.

(7) That the exposure to high concentration of dissolved oxygen with a super-stratum of pure oxygen at atmospheric pressures and under small pressure is not harmful is inferred from the small number of fish lost and from the length of time for which a majority of the fish survived the experiment.

(8) The data presented here suggest that they may be applicable to the problem of handling fish during transportation.

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Glaciation.

For those interested in glaciation, especially in the New England region, this little book shows the possibilities of what can be compiled and observed by careful, close attention to a small area. The book is worth reading because of the completeness of the information it contains, although I can find no conclusions drawn from all this information. The general get up of the book is good with the exception of the two maps, both of which are crowded on their respective pages, allowing a margin of a scant one-eighth inch. The same may be said of the figures on page 19. I also note that the word schist is misspelled in several places.

WILLARD BERRY.

Alpine Zone of Mt. Washington Range, by Ernst Antevs. viii + 118 pp. Merrill & Weber Co., 1932. To be ordered from Bertha B. Smith, 28 Beacon Ave., Auburn, Me.

A COMPARISON BETWEEN THE SPECTROPHOTOMETRIC AND BIOLOGICAL ASSAY FOR THE VITAMIN A CONTENT OF FISH-LIVER OILS.

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A standardized method of color analysis for Vitamin A (1) by the antimony trichloride reaction has been applied to commercial samples of fish-liver oils. A comparison between the spectrophotometric values and those obtained by biological assay would indicate that there is a considerable error in the antimony trichloride test.

The fish-liver oils which were used in this series of experiments were furnished by the Parke Davis and Company, the Abbott Laboratories, the Eli Lilly Company, the E. R. Squibb and Sons, the Mead Johnson Company and the Upjohn Laboratories. Each of these companies gave feeding tests on their oils so that data could be obtained on the relative values and agreement between the chemical and biological assays. The Parke Davis and Company, through the courtesy of Dr. A. D. Emmett, furnished in addition, colormetric data on their oils as obtained by the Carr Price method, which were made over a period of several years. The data furnished a check on the relation between the colormetric and spectrophotometric methods, as well as the influence of time on these oils. All of the samples used in the tests described in this paper were kept in a refrigerator at 5° C.

The oils have been divided into two sets, (a) those giving with the antimony trichloride reagent an absorption band at 608 mμ (Table I) and (b) those giving an absorption band at 622 mμ (Table II). Ordinary "cod-liver" oil sold in pharmaceutical trade is made from the genus of fish known as the *gadus morrhua*, and generally not exclusively from the cod fish. This group includes the cod, haddock, hake and pallock. All of the oils in Table I were designated as cod-liver oils. They included both domestic and foreign oils. Records on some of the oils would indicate that they had been in storage for some time, while others were quite fresh. There was no apparent relationship between the variations in the color test—biological assay values and the age or source of the oil.

In certain cases where a marked difference was shown between the values obtained from the two methods of assay, both types of assays were repeated and confirmed this difference.

TABLE I.

COMPARISON BETWEEN THE SPECTROPHOTOMETRIC AND BIOLOGICAL ASSAY DATA ON COD-LIVER OILS (BASED ON THE ABSORPTION BAND AT 608 mμ).

Company	Sample No.	Biological Assay	Spectrophotometric Assay (Referred to Sample No. 11 as a Standard)	
I.....	1	573	590	
	2	500	780	
	3	1000	640	
	4	500	850	
	5	625	800	
II.....	6	625–500	590	
	7	400–500	690	
	8	450–550	750	
	9	600–666	770	
	10	625+	920	
III.....	11	1000	1000	
	12	3200	2790	
	13	800	820	
	14	700	670	
	15	600	670	
	16	1900	1060	
	17	800	590	
	18	1350	1730	
	19	1800	1930	
	20	1900	1500	
	21	1000	1190	
IV.....	22	1000	1170	
	23	1000	850	
	24	4000	5300	
	25	4500	5500	
	26	1300	1300	
				(½ Spectrophotometric Values)
V.....	27	650	1220*	610*
	28	700	1520	760
	29	500	1100	550
	30	650	1500	750
VI.....	31	1200	2600	1300
	32	500	1100	550
	33	850	1700	850

*NOTE.—In Groups V and VI it would seem necessary to multiply the spectrophotometric values by a factor of ½ to make the values obtained by this method agree with those obtained by the biological assay.

In Table II are recorded the results obtained from halibut-liver oils. Here, again, there was found to be a wide variation between the two methods of analysis. These oils were for the most part concentrates of the unsaponifiable fraction obtained from the crude oil. In certain cases (samples 34 and 35) the concentrated oils were diluted with sesame oil.

No explanation is offered for the necessity of this factor, of ½, as applied in groups V and VI in Table I, though a

TABLE II.

Comparison Between the Spectrophotometric and Biological Assay Data on Halibut-Liver Oils (Based on the Absorption Band at 622 mμ).

Company	Sample No.	Biological Assay	Spectrophotometric Assay (Referred to the Height of the Extinction Coefficient of No. 11, Table I, as a Standard)
VII	34	500	650
	35	2500	2310
	36	30000	37800
VIII	37	37,500–50,000	64700
	38	37,500–50,000	113800
	39	25,000–37,500	78400
	40	13000	29300
IX	41	5000	17000
	42	32500	94800
	43	5000	3800
	44	17500	73200
X	45	62500	84000

justification for its use can be seen in certain groups of oils, where all the members had to be corrected by the same factor. Both the Spectrophotometric and biological assays were repeated on certain of these oils, the results of which only confirmed the previously obtained values. Undoubtedly these variations, since they are so consistent, are due to some cause other than errors of observation, and may be ascribed to some inhibitor, activator or to alteration in the structural nature of the chromophore.

In Table II are presented data on the halibut-liver oils, which gave a band at 622 mμ, or some 14 mμ further towards the red as compared with the color obtained from the cod-liver oils with antimony trichloride. This shift has been reported

by Morton (2). Heilbron, Gillman and Morton (3) suggest that the substance giving the 572 mμ (our 578 mμ) band is an ester and the substance producing the 583 mμ band (which appears at the same time as the 622 mμ band) is an alcohol derived from the former substance by saponification. None of the oils in Table II gave the 608 mμ band as far as could be observed by inspection of the absorption bands, and no amount of dilution with $CHCl_3$ shifted the position of the 622 mμ band. There was a weaker band at 705 mμ which apparently accompanies the 622 mμ band.

The existence of vitamin-containing oils giving markedly different absorption bands (608 mμ and 622 mμ) leads one to postulate the theory that Vitamin A is not a definite chemical compound, but that the physiological effect produced by the so-called "A" vitamin is due to any one of a series of closely related compounds, which must have certain structures in common. As already stated Morton suggests that this difference may be as simple as that between an alcohol and an ester. However, the fact that carotene fades in a manner similar to the fading of fish oils suggests a more complicated mechanism.

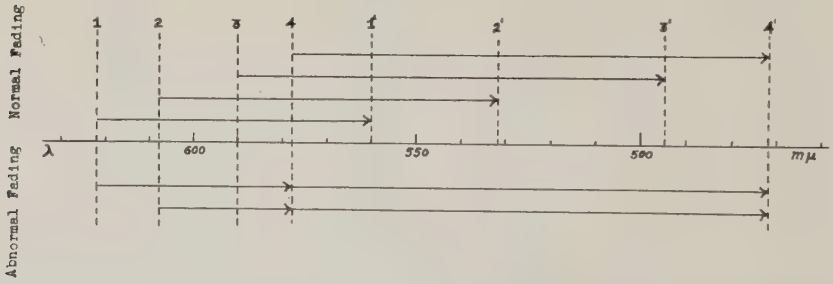

FIGURE 1. Graphical representation of the changes in absorption bands on fading. 1 to 1′ representing the change from 622 mμ to 560 mμ; 2 to 2′ from 608 to 532 mμ; 3 to 3′ from 590 to 495 mμ; and 4 to 4′ from 578 to 472 mμ. The abnormal fading represented below the wave length scale indicates an apparent change from the original chromogen to the 578 form and then fading of the 578 to the final 472 form. This abnormal fading is slight in the case of the 608 mμ chromogen but is the principal reaction in the case of the 622 mμ chromogen.

Following the diagrammatic outline (Figure 1) for the band shifts:

(a) It can be seen that the cod-liver oils producing a band at 608 mμ fade to give a band at 532 mμ (2 to 2′) and those giving a band at 578 mμ fade to 472 mμ (4 to 4′). In case a rather concentrated solution was used so as to give only the 608 mμ and no 578 mμ band, faded bands were observed at both 532 mμ and 472 mμ (2 to 4′). This would indicate that there is a shift from 608 to 578 together with the shift

from 608 to 532 mμ, the latter being more rapid than the former. If the amount of chromogen is unusually large, some 608 changes to 578 which undergoes its normal fading reaction to 472 mμ. In this case just considered, no 578 mμ band appears because the change from 578 to 472 mμ is more rapid than that from 608 to 578 mμ.

(b) The above hypothesis is supported by the fact that if more chromogen is present, both 608 and 578 mμ bands are produced in the blue solution, and both 532 and 472 mμ bands appear in the faded solution. In this case, the 578 mμ band is entirely extinct before the 608 mμ band is extinct, indicating that the amount of chromogen has been reduced to the amount present in case (a) above. These considerations also explain why solutions of oil dilute enough to produce no 578 mμ bands must be used to obtain spectrophotometric values of the 608 mμ bands which are directly proportional to the amount of oil used.

(c) In the case of the halibut-liver oils, a band is produced at 622 mμ, which corresponds to the 608 mμ band produced in ordinary oils. Since the predominate band in the faded solution is at 472 mμ, most of the 622 chromogen must change to the 578 chromogen, which then undergoes its normal fading reaction. Other bands appear in the faded solutions of these oil concentrates at 545, 560, and 580 mμ, only the one at 472 being strong and well-defined.

(d) If the solution of cod-liver oil is made up so that only the 578 mμ (and no 608 mμ) band is produced, the only absorption band in the faded solution is at 472 mμ (4 to 4′).

(e) Carotene gives a band with the $SbCl_3$ reagent at 590 mμ, which fades to give a band at 488 mμ. (Fig. 1, 3 to 3′).

The linear equation ($y = mx + b$) can be used to predict the position of the absorption bands in the faded solutions of these substances.

Let y = band shift expressed in cm^{-1};
x = original position of the band expressed in cm^{-1}; and
m and b are constants to be determined.

Using the band shifts, 578 to 472 mμ and 608 to 532 mμ, the constants m and b can be found, and their values are 1.797 and −27,215 respectively. These values can be substituted in the equation, and the value of "x" determined for each substance, from which the position of the faded band can be calulated:

Original band ("x")	Value of "y"	Faded Band Calculated	Faded Band Observed
(a) Cod-liver oil			
608 mμ	2,348 cm^{-1}	532 mμ	532 mμ
578 mμ	3,879 cm^{-1}	472 mμ	472 mμ
(b) Halibut-liver oil			
622 mμ	1,683 cm^{-1}	563 mμ	560 mμ
(c) Carotene			
590 mμ	5,249 cm^{-1}	495 mμ	488 mμ

The values obtained are all within the limits of experimental accuracy.

It is to be noted that if the "chromogen" in the oil concentrates reacts in what we might term normal fading we would expect to obtain a band at about 560 mμ, where a weak band was observed.

The mechanism of the fading of the 622 mμ band to a band at 560 mμ appears to be perfectly normal and subject to the same explanations that might be applied to the fading of the other solutions. As indicated by the strength of the 560 mμ band as compared with that of the 472 mμ band formed in the same solution only a small part of the chromogen undergoes normal fading, while the principal reaction is the conversion of the chromogen giving a band at 622 mμ into one with a band at 580 mμ which in turn fades to the substance with a band at 472 mμ. Due to the similarity of the bands there is very little doubt but that the 580 mμ band, which was not very well defined, is the same as the 578 mμ band. If, as is apparently the case, the speed of the fading reaction of the chromogen with a band at 578 mμ to one with a band at 472 is equal to, or greater than the speed of the reaction converting the chromogen with a band at 622 mμ to one with a band at 578 mμ, little, if any, indication of the presence of the 578 mμ band would be apparent in the fading solution. It follows then that the chromogen with a band at 622 mμ must either be more stable than the chromogen with a band at 608 mμ, with regard to what we have termed the normal fading (see Fig. 1) or less stable than the chromogen with the 608 mμ band with regard to conversion to the form with a band at 578 mμ, since the principal reaction on the fading of the chromogen with a band at 622 mμ is the formation of a substance with a band at 472 mμ rather than at 560 mμ. Observations on the rate of fading of the halibut-liver oil and the cod-liver oil colors showed that the colors formed by the antimony trichloride reaction with the halibut-liver oils were much more permanent than those colors formed with the cod-liver oils.

Table III is presented to show the agreement over a period of years of the spectrophotometric and colorimetric values. There is very little aging effect over the period indicated.

One of the cod-liver oils was found to be highly flavored with peppermint oil. The spectrophotometric readings on this sample were not proportional to the amount of oil used unless the oil was comparatively dilute, (about a 3% solution in $CHCl_3$). The values obtained by this procedure agreed with

the animal assay. It was subsequently found that peppermint oil in the presence of certain reagents such as sulfuric acid, acetic anhydride, etc., and to a lesser extent with antimony trichloride, gives an absorption band similar to that produced by the fish-liver oils. Perhaps of even more significance is the fact that in the standard color reaction for peppermint oil (5) a blue color is produced with absorption bands at 622, 580, 505 and a weak band at 705 mμ. It was the presence of

TABLE III.

Comparison Between the Colorimetric and Spectrophotometric Assay of Cod-Liver Oils.

Sample No.	Date of Observation			
	Colorimetric			Spectrophotometric
	1928	1928	1931	1931
11	10	9	12	10
12	19	22	27	27.9
13	10	9	10	8.2
14	5.9	4.7	7.5	6.7
15	6.5	6.5	7.5	6.7
16	10	9	10	10.6
17	5.9	5.9	3.9	5.9
18	15	15	16	17.3
19	.....	15	16	19.3
20	.....	13	13	15.0
21	.....	12	12	11.9
27	10	7.5	10	12.2
28	.....	.....	13	15.2
29	.....	9	10	11.0
30	.....	12	13	15.0

this latter weak band in the more stable and concentrated color solutions of the peppermint oils that prompted a re-examination of the blue color produced with the halibut-liver oils, with the discovery of a similar band at 705 mμ. (4) In some peppermint oil solutions, particularly those to which mineral acids were added, a band was produced at 605 mμ instead of at 622 mμ. The conditions under which either of these two bands (605 mμ or 622 mμ) might be produced were not determined.

The nature of this blue material in the peppermint oil test is a matter of uncertainty. It has been reported to be azulene, which in turn is said to be produced by the oxidation of cadinene (a member of the sesquiterpene family). An investigation of the green color produced by cadinene, obtained

by distillation of cade oil, showed bands at 605 and 670 mμ, while a sample of German Chamomile oil in chloroform (obtained from Fritschie Bros. who obtained it from Schimmel and Co.) (the source of the material used by Ruczika in his study of azulene (6)) which was bright bule in color gave sharp bands at 608 and 660 mμ. Careful distillation of oil of peppermint produced a fraction, B. P. 60-65° (2-3 mm.) in which practically all of the color forming material is to be found. The blue solutions give a most brilliant red fluorescence.

Summary.

A method for the testing of oils for vitamin A by the use of the $SbCl_3$ reagent has been applied to a number of commercial samples with some agreement between the colorimetric and biological assay values within certain groups of samples.

Differences which did occur were consistent in the samples from any one laboratory or for a a given set of samples.

The nature of the absorption bands produced by $SbCl_3$ on cod-liver oils, halibut-liver oils, and carotene and the resulting faded bands would indicate that several closely allied compounds may be responsible for both the color reactions and the vitamin properties.

Oil of peppermint contains a color-forming compound which has to a rather marked degree the same type of chromogen as is to be found in certain oils known to contain vitamin A.

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Geology.

This book, gotten out in the usual McGraw-Hill style, may well fill a place in the text-book field that has been neglected, i. e., "one-semester beginning course for college students," as the authors say in the preface. The general arrangement and order of presentation would seem to be that which would do very well in such a short course. The text is not over-burdened with needless detail, although some of the words are the less common ones of geologic literature. The illustrations are from many sources; numerous of them are new to textbooks. While I cannot recommend it for longer courses, it should find use in those places where Geology only receives one semester or one quarter of the time of a student—Willard Berry.

Geology, by W. H. Emmons, G. A. Thiel, C. R. Staufer, and I. S. Allison. xii + 514 pp. New York, The McGraw-Hill Book Co., 1932.

A STATISTICAL SURVEY OF OHIO WINTER BIRD LIFE.

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Ornithology was prominent among the biological sciences to first receive any great attention in Ohio. This early developed interest has continued down to the present day and several hundred articles have been published relating to various phases of Ohio bird life. One subject which has been almost entirely neglected until recently, is that which concerns the frequency of occurrence and the relative abundance of Ohio winter birds.

Although ornithologists in all sections of the country have co-operated for 32 years in an annual winter bird census sponsored by Bird Lore Magazine, no attempt has yet been made to assemble the large mass of Ohio data which has accumulated and to apply it to the solution of problems which concern the numbers and distribution of our winter birds.

In the past few years the writers have carefully compiled all the data from Ohio censuses and in the following pages have attempted to present this information in a usable form along with certain interpretations. Little attempt has been made to evaluate the data. They are presented merely for whatever value they may have. Much additional information has also been prepared and placed on file, including for each species the number of individuals observed each year, the average number per census and graphs showing any actual or apparent yearly fluctuations in numbers as shown by the census counts. It is planned to continue this compilation from year to year. These data are available for use by anyone interested in further research on the subject.

All census enumerators have followed a standard plan recommended by Bird Lore since 1900. The usual procedure

is to begin the census field work as early as possible after sunrise, carefully covering an area not to exceed 15 miles in diameter. The observer or party of observers (sometimes several parties working separately), enumerates all birds seen on the trip and tabulates the totals for each species, total number of species and total number of individuals seen. Census co-operators usually spend the full day (seldom less than one-half day) in the field and cover all available area types to get

FIG. 1. Map of Ohio showing census localities.

as nearly as possible a representative picture of the bird life of their section. Records of the temperature, general weather conditions, miles traveled, and various miscellaneous data are sent to Bird Lore. Censuses are taken on any day from December 21 to December 27, and the results are published in the January-February issue of the magazine each year.

Various obvious limitations occur in the use of this compiled information. The data must be properly interpreted and corrected for a number of factors as will be pointed out later in this article. The census observers are, for the most part,

well trained and competent to make counts and correct identifications. The quality of the censuses has steadily increased from year to year as the work became of increasing interest and the co-operators developed a technique for an efficient and more thorough enumeration. The number of species observed per census has also increased. This is believed to be largely due to the increasing interest, greater effort expended,

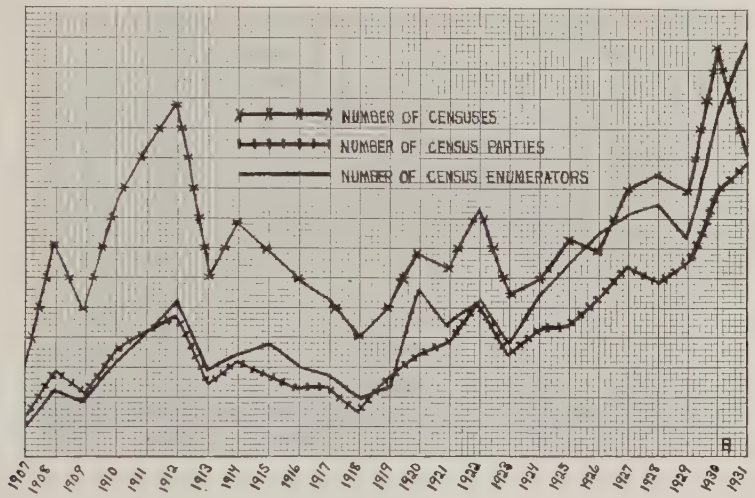

FIG. 2. Graph showing the relative variation from year to year in the number of censuses taken, the number of census parties (sometimes from 2 to 6 census parties combine their records in one census report), and the number of census enumerators. (For actual values of each, see text.) Any graphs made from census data to show the fluctuation in numbers from year to year of a species, should be superimposed upon this graph to aid in proper evaluation of the statistics. This will assist in determining whether certain apparent fluctuations indicate actual changes in numbers.

and the fact that many censuses formerly taken by one or two observers are now secured through the efforts of a group of 5 to 20 enumerators, frequently working in several parties and covering much larger areas than formerly. Exactly half of the censuses by more than two observers have been taken from 1925 to 1931, and half of those taking censuses have participated since 1924.

A summary of the censuses taken to date is shown in Table I, on the following page.

TABLE I.

Year	Number of Censuses Taken	Number of Census Parties	Number of Census Cooperators	Total Species in Whole State	Average Number of Species per Census	Average Number of Species per Census—Period Av'ge
1900	1	1	1	17	14	
1901	2	2	2	17	10.5	
1902	4	4	4	22	13	1900–1906
1903	3	3	5	26	19.6	
1904	3	3	5	27	16.6	15.6
1905	2	2	3	24	16	
1906	2	2	3	27	19.5	
1907	5	5	8	29	12.2	
1908	15	15	18	47	13.8	1907–1911
1909	10	10	15	36	13.6	
1910	17	18	24	40	11.4	12.8
1911	21	21	29	41	13.1	
1912	24	24	38	57	15.9	
1913	12	12	20	48	16.8	1912–1916
1914	16	16	25	47	17.3	
1915	14	14	28	51	14.5	16.1
1916	12	12	22	50	16.0	
1917	11	12	19	50	16.3	
1918	8	8	14	44	14.3	1917–1921
1919	10	13	18	48	16.4	
1920	14	17	41	57	17.7	16.8
1921	13	19	32	60	19.5	
1922	17	26	39	61	23.6	
1923	11	16	27	69	23.1	1922–1926
1924	12	21	38	70	25.6	
1925	15	22	46	73	25.2	24.2
1926	14	26	54	74	23.8	
1927	18	32	58	84	26.3	
1928	19	29	57	72	24.8	1927–1931
1929	18	31	52	71	25.6	
1930	27	45	81	81	21.4	25.7
1931	21	49	98	108	30.8	
Total	392	529	934	.....	18.8	

Species per census 1900–1931 = 18.8. Species per census 1922–1931 = 25.0.

199 censuses taken by single observers averaged in number of species.....18.0
108 censuses taken by two observers averaged in number of species.....18.7
30 censuses taken by three observers averaged in number of species.....18.7
18 censuses taken by four observers averaged in number of species.....20.8
10 censuses taken by five observers averaged in number of species.....25.0
6 censuses taken by six observers averaged in number of species.....29.0
3 censuses taken by seven observers averaged in number of species.....37.3
2 censuses taken by eight observers averaged in number of species.....26.0
4 censuses taken by nine observers averaged in number of species.....32.0
2 censuses taken by ten observers averaged in number of species.....43.0
3 censuses taken by eleven observers averaged in number of species.....40.3
3 censuses taken by twelve observers averaged in number of species.....46.3

In addition, single censuses by 13, 15, 16 and 18 observers secured 47, 34, 40 and 50 species respectively.

The following species of Ohio winter birds have been reported in the Christmas Censuses published in Bird Lore from 1901 to 1932, including a total of 392 censuses taken during the 32 years from 1900 to 1931, inclusive. A total of 934 co-operators (about 500 different persons), have taken part in these censuses, from 79 localities in 49 Ohio counties. In all, a total of 133 species and 222,825 individuals have been recorded. Nomenclature and arrangement is that of the American Ornithologists' Union Check List (1931) 4th Edition.

1. Common Loon. *Gavia immer immer* Brunnich.
2. Holboell's Grebe. *Colymbus grisenga holboelli* Reinhardt.
3. Horned Grebe. *Colymbus auritus* Linnaeus.
4. Pied-billed Grebe. *Podilymbus podiceps podiceps* Linnaeus.
5. Double-crested Cormorant. *Phalacrocorax auritus auritus* Lesson.
6. Great Blue Heron. *Ardea herodias herodias* Linnaeus.
7. Black-crowned Night Heron. *Nycticorax nycticorax hoactli* Gmelin.
8. American Bittern. *Botaurus lentiginosus* Montague.
9. Common Canada Goose. *Branta canadensis canadensis* Linnaeus.
10. Lesser Snow Goose. *Chen hyperborea hyperborea* Pallas.
11. Common Mallard. *Anas platyrhynchos platyrhynchos* Linnaeus.
12. Common Black Duck. *Anas rubripestristis* Brewster. (Including the Red-legged Black Duck, *Anas rubripes rubripes* Brewster.)
13. Gadwall. *Chaulelasmus streperus* Linnaeus.
14. Baldpate. *Mareca americana* Gmelin.
15. American Pintail. *Dafila acuta tzitzihoa* Vieillot.
16. Green-winged Teal. *Nettion carolinense* Gmelin.
17. Shoveller. *Spatula clypeata* Linnaeus.
18. Redhead. *Nyroca americana* Eyton.
19. Ring-necked Duck. *Nyroca collaris* Donovan.
20. Canvasback. *Nyroca valisineria* Wilson.
21. Lesser Scaup. *Nyroca affinis* Eyton.
22. American Goldeneye Duck. *Glaucionetta clangula americana* Bonaparte.
23. Bufflehead. *Charitonetta albeola* Linnaeus.
24. Oldsquaw Duck. *Clangula hyemalis* Linnaeus.
25. White-winged Scoter. *Melanitta deglandi* Bonaparte.
26. Ruddy Duck. *Erismatura jamaicensis rubida* Wilson.
27. Hooded Merganser. *Lophodytes cucullatus* Linnaeus.
28. American Merganser. *Mergus merganser americanus* Cassin.
29. Red-breasted Merhanser. *Mergus serrator* Linnaeus.
30. Turkey Vulture. *Cathartes aura septentrionalis* Wied.
31. Black Vulture. *Coragyps atratus atratus* Meyer.
32. Eastern Goshawk. *Astur atricapillus atricapillus* Wilson.
33. Sharp-shinned Hawk. *Accipiter velox* Wilson.
34. Cooper's Hawk. *Accipiter cooperi* Bonaparte.
35. Eastern Red-tailed Hawk. *Buteo borealis* Gmelin.
36. Northern Red-shouldered Hawk. *Buteo lineatus lineatus* Gmelin.
37. American Rough-legged Hawk. *Buteo lagopus s. johannis* Gmelin.
38. Golden Eagle. *Aquila chrysaetos canadensis* Linnaeus.
39. Northern Bald Eagle. *Haliaetus leucocephalus alascanus* Townsend.
40. Marsh Hawk. *Circus hudsonius* Linnaeus.
41. Duck Hawk. *Falco peregrinus anatum* Bonaparte.
42. Eastern Pigeon Hawk. *Falco columbarius columbarius* Linnaeus.
43. Eastern Sparrow Hawk. *Falco sparverius sparverius* Linnaeus.
44. Ruffed Grouse. *Bonasa umbellus umbellus* Linnaeus.
45. Hungarian or European Partridge. *Perdix perdix perdix* Linnaeus.
46. Eastern Bobwhite. *Colinus virginianus virginianus* Linnaeus.
47. Ring-necked Pheasant. *Phasianus colchicus torquatus* Gmelin.
48. American Coot. *Fulica americana americana* Gmelin.

49. Kildeer. *Oxyechus vociferus vociferus* Linnaeus.
50. American Woodcock. *Philohela minor* Gmelin.
51. Wilson's Snipe. *Capella delicata* Ord.
52. Herring Gull. *Larus argentatus smithsonianus* Coues.
53. Ring-billed Gull. *Larus delawarensis* Ord.
54. Franklin's Gull. *Larus pipixcan* Wagler.
55. Bonaparte's Gull. *Larus philadelphia* Ord.
56. Easter Mourning Dove. *Zenaidura macroura carolinensis* Linnaeus.
57. Barn Owl. *Tyto alba pratincola* Bonaparte.
58. Eastern Screech Owl. *Otus asio naevius* Gmelin.
59. Great Horned Owl. *Bubo virginianus virginianus* Gmelin.
60. Snowy Owl. *Nyctea nyctea* Linnaeus.
61. Barred Owl. *Strix varia varia* Barton.
62. Long-eared Owl. *Asio wilsonianus* Lesson.
63. Short-eared Owl. *Asio flammeus flammeus* Pontoppidan.
64. Saw-whet Owl. *Cryptoglaux acadica acadica* Gmelin.
65. Eastern Belted Kingfisher. *Megaceryle alcyon alcyon* Linnaeus.
66. Northern Flicker. *Colaptes auratus luteus* Bangs.
67. Northern Pileated Woodpecker. *Coephloeus pileatus abieticola* Bangs.
68. Red-bellied Woodpecker. *Centurus carolinus* Linnaeus.
69. Red-headed Woodpecker. *Melanerpes erythrocephalus* Linnaeus.
70. Yellow-bellied Sapsucker. *Sphyrapicus varius varius* Linnaeus.
71. Eastern Hairy Woodpecker. *Dryobates villosus villosus* Linnaeus.
72. Northern Downy Woodpecker. *Dryobates pubescens medianus* Swainson.
73. Eastern Phoebe. *Sayornis phoebe* Latham.
74. Northern Horned Lark. *Otocorus alpestris alpestris* Linnaeus.
75. Prairie Horned Lark. *Otocorus alpestris praticola* Henshaw.
76. Northern Blue Jay. *Cyanocitta cristata cristata* Linnaeus.
77. Eastern Crow. *Corvus brachyrhynchos brachyrhynchos* Brehm.
78. Black-capped Chickadee. *Penthestes atricapillus atricapillus* Linnaeus.
79. Carolina Chickadee. *Penthestes carolinensis carolinensis* Audubon.
80. Tufted Titmouse. *Baeolophus bicolor* Linnaeus.
81. White-breasted Nuthatch. *Sitta carolinensis carolinensis* Latham.
82. Red-breasted Nuthatch. *Sitta canadensis* Linnaeus.
83. Brown Creeper. *Certhia familiaris americana* Bonaparte.
84. Eastern Winter Wren. *Nannus hiemalis hiemalis* Vieillot.
85. Bewick's Wren. *Thryomanes bewicki bewicki* Audubon.
86. Carolina Wren. *Thryothorus ludovicianus ludovicianus* Latham.
87. Prairie (Long-billed) Marsh Wren. *Telmatodytes palustris dissaeptus* Bangs.
88. Eastern Mockingbird. *Mimus polyglottos polyglottos* Linnaeus.
89. Brown Thrasher. *Toxostoma rufum* Linnaeus.
90. Eastern Robin. *Turdus migratorius migratorius* Linnaeus.
91. Wood Thrush. *Hylocichla mustelina* Gmelin.
92. Eastern Hermit Thrush. *Hylocichla guttata faxoni* Bangs & Penard.
93. Eastern Bluebird. *Sialia sialis sialis* Linnaeus.
94. Eastern Golden-crowned Kinglet. *Regulus satrapa satrapa* Lichtenstein.
95. Eastern Ruby-crowned Kinglet. *Corthylio calendula calendula* Linnaeus.
96. American Pipit. *Anthus spinoletta rubescens* Tunstall.
97. Bohemian Waxwing. *Bombycilla garrula pallidiceps* Reichnow.
98. Cedar Waxwing. *Bombycilla cedrorum* Vieillot.
99. Northern Shrike. *Lanius borealis borealis* Vieillot.
100. Migrant Shrike. *Lanius ludovicianus migrans* Palmer.
101. Starling. *Sturnus vulgaris vulgaris* Linnaeus.
102. Orange-crowned Warbler. *Vermivora celata* Say.
103. Myrtle Warbler. *Dendroica coronata* Linnaeus.
104. Western Palm Warbler. *Dendroica palmarum palmarum* Gmelin.
105. Eastern Meadowlark. *Sturnella magna magna* Linnaeus.
106. Eastern Red-wing. *Agelaius phoeniceus phoeniceus* Linnaeus.
107. Rusty Blackbird. *Euphagus carolinus* Muller.
108. Bronzed Grackle. *Quiscalus quiscula aeneus* Ridgeway.
109. Eastern Cowbird. *Molothrus ater ater* Boddaert.
110. Eastern Cardinal. *Richmondena cardinalis cardinalis* Linnaeus.
111. Indigo Bunting. *Passerina cyanea* Linnaeus.

112. Eastern Evening Grosbeak. *Hesperiphona vespertina vespertina* Cooper.
113. Eastern Purple Finch. *Carpodacus purpureus purpureus* Gmelin.
114. Canadian Pine Grosbeak. *Pinicola enucleator leucura* Muller.
115. Common Redpoll. *Acanthis linaria linaria* Linnaeus.
116. Northern Pine Siskin. *Spinus pinus pinus* Wilson.
117. Eastern Goldfinch. *Spinus tristis tristis* Linnaeus.
118. Red Crossbill. *Loxia curvirostra pusilla* Gloger.
119. White-winged Crossbill. *Loxia leucoptera* Gmelin.
120. Red-eye Twohee. *Pipilio erythrophthalmus erythrophthalmus* Linnaeus.
121. Eastern Vesper Sparrow. *Pooecetes gramineus gramineus* Gmelin.
122. Slate-colored Junco. *Junco hyemalis* Linnaeus.
123. Eastern Tree Sparrow. *Spizella arborea arborea* Wilson.
124. Eastern Chipping Sparrow. *Spizella passerina passerina* Beckstein.
125. Eastern Field Sparrow. *Spizella pusilla pusilla* Wilson.
126. White-crowned Sparrow. *Zonotrichia leucophrys leucophrys* Forster.
127. White-throated Sparrow. *Zonotrichia albicollis* Gmelin.
128. Eastern Fox Sparrow. *Passerella iliaca iliaca* Merrem.
129. Swamp Sparrow. *Melospiza georgiana* Latham.
130. Mississippi Song Sparrow. *Melospiza melodia beata* Bangs.
131. Lapland Longspur. *Calcarius lapponicus lapponicus* Linnaeus.
132. Eastern Snow Bunting. *Plectrophenax nivalis nivalis* Linnaeus.

The following additional species have been reported one or more times by various census enumerators but have been omitted from the above list because there is some question as to their proper identification and occurrence during the Christmas season: Green Heron (*Butorides virescens virescens* Linn.), Broad-winged Hawk (*Buteo platypterus platypterus* Vieillot), Greater Scaup (*Nyroca marila* Linn.), and Pomarine Jaeger (*Stercorarius pomarinus* Temm.). The Chickadees recorded in the censuses were frequently incorrectly listed as to species, or merely listed without determination of species. Records of Chickadees in the enumeration have been divided between the two species occurring in Ohio according to the present known local range of each in winter. Birds listed merely as Horned Larks have been referred to the Northern Horned Lark, though a number of these were probably Prairie Horned Larks and perhaps a few, Hoyt's Horned Larks. A number of unidentified ducks, hawks, owls and other birds have been disregarded in the enumeration.

The total number of accepted species listed on the census reports totals 133. Twenty-nine additional species are known to have occurred in Ohio during the winter season, but have never been observed during a Christmas census, making a total of 162 species, (see Annotated Check-list of Ohio Winter Birds, L. E. Hicks, 1932). These are as follows:

Gannet. *Moris bassana* Linnaeus.
Blue Goose. *Chen caerulescens* Linnaeus.
Red-legged Black Duck. *Anas rubripes rubripes* Brewster.
Greater Scaup. *Nyroca marila* Linnaeus.

Barrow's Golden-eye. *Glaucionetta islandica* Gmelin.
American Eider. *Somateria mollissima dresseri* Sharpe.
King Eider. *Somateria spectabilis* Linnaeus.
Surf Scoter. *Melanitta perspicillata* Linnaeus.
Broad-winged Hawk. *Buteo platypterus platypterus* Vieillot.
Osprey. *Pandion haliaetus carolinensis* Gmelin.
Black Gyrfalcon. *Falco rusticolus obsoletus* Gmelin.
Prairie Chicken. *Tympanuchus cupido americanus* Reichenbach.
Virginia Rail. *Rallus limicola limicola* Vieillot.
Florida Gallinule. *Gallinula chloropus cachinnans* Bangs.
Iceland Gull. *Larus leucopterus* Vieillot.
Great Black-backed Gull. *Larus marinus* Linnaeus.
Atlantic Kittewake. *Rissa tridactyla tridactyla* Linnaeus.
Brunnich's Murre. *Uria lomvia lomvia* Linnaeus.
American Hawk Owl. *Surnia ulula caparoch* Muller.
Great Gray Owl. *Scotiaptex nebulosa nebulosa* Forster.
Arctic Three-toed Woodpecker. *Picoides arcticus* Swainson.
Hoyt's Horned Lark. *Otocoris alpestris hoyti* Bishop.
Pine Warbler. *Dendroica pinus pinus* Wilson.
Northern Yellowthroat. *Geothlypis trichas brachidactyla* Swainson.
English Sparrow. *Passer domesticus domesticus* Linnaeus. (Not enumerated.)
Baltimore Oriole. *Icterus galbula* Linnaeus.
Savannah Sparrow. *Passerculus sandwichensis savanna* Wilson.
Grasshopper Sparrow. *Ammodramus savannarum australis* Maynard.
Smith's Longspur. *Calcarius pictus* Swainson.

The results of the following tabulated data, if properly interpreted, will contribute answers to many important questions concerning Ohio winter bird life, especially those relating to frequency of occurrence and the relative abundance of species present. Individuals actually recorded on any field trip never give an exactly accurate picture of the total bird population present. Corrections must be made for a number of obvious factors. Human tendencies and weaknesses as well as the general habits of the species must be taken into consideration. Certain species will be recorded in numbers not indicative of their true abundance because of:

(1) having loud, clear, frequently uttered notes or songs, making it possible to identify and record the species from a considerable distance, resulting in an actual survey of a larger area for some species than for others.

(2) excessive activity, curiosity and tameness, large size, and conspicuous plumage.

(3) having habits which make the species observable in readily accessible places and against backgrounds which make the bird conspicuous. Example, ground birds vs. arboreal species.

(4) habits of concentration about water, cover, special habitats or where certain foods abound, making it possible to record the species in excess of its true numbers.

(5) activity and presence in accessible areas or locations during hours of the census. For example, according to compiled data, 725

Sparrow Hawks were recorded on 205 or 52% of a total of 392 censuses taken, while only 157 Screech Owls were recorded on 98 or 25% of the censuses and were not checked on the remaining 235. Because of its nocturnal and secretive habits, the latter species is never recorded in proportion to its true numbers and there is good reason to believe that the Screech Owl actually exceeds the Sparrow Hawk in numbers in most Ohio localities during winter.

(6) various historical factors involved. Many species have been decidedly increasing or decreasing in numbers. The figures indicated do not show the present status but the average for a 32-year period. Since the census work began, one species, the Ring-necked Pheasant, has been widely introduced, now occurs in every county and is common to abundant in more than half of the state. Another game bird, the Hungarian Partridge, has been introduced generally and has become common or abundant in about 20 northwestern counties, occurring locally in certain other sections.

The European Starling was first reported from Ohio in January, 1916, from West Lafayette, Coshocton County. The writers have observed the species every year since 1918, making a special study of its first appearances and its subsequent invasion of the state. Records of the species became frequent from 1920 to 1923, and the birds were abundant from 1924 to 1928, spreading westward and southwestward to every county. Starlings were first recorded on a Christmas census in 1920. One report in 1922 gave 14 birds; 2 in 1923, 61; 4 in 1924, 56; 5 in 1925, 177; 12 in 1927, 455; 12 in 1928, 1,218; 13 in 1929, 2,557; 21 in 1930, 2,174; and 27 censuses in 1931 reported 8,570 birds.

Although, of course, not so indicated in the table because of its short occurrence in the state, the Starling is now one of the five commonest, and probably the most common bird in winter, outnumbering on reports of the last five years, all other species except the Crow.

(7) flocking tendencies on the part of certain species. This is a particularly important factor. Flocking and gregarious species always give the impression of being much more abundant than they really are and will be recorded in greater than their actual relative numbers on census lists because of their conspicuousness, ready identification and concentration. This occurs in spite of the fact that most observers tend to greatly underestimate the size of bird flocks, where actual counts cannot be taken.

Of the first ranking 25 species on the basis of individual numbers, 17 are flocking species. Of the first ranking 25 on the basis of number of censuses reporting the bird, only 10 are of the flocking type. In determining the actual status of a species and its true abundance, there is good evidence to show that not only the number of individuals recorded, but also the regularity of occurrence, should be taken into consideration. Where both of these indicators of abundance are used, the list of the first 25 includes 12 gregarious species.

In the table below, column No. 1 ranks the 132 birds reported on censuses by taking into equal consideration the number of individuals reported and the number of times occurring on censuses. Column 2 gives first the number of censuses on which each species has been listed out of a total of 392 taken from 1900 to 1931, and secondly, the number of years in which each species was recorded. Column 3 shows the total number of individuals of each species reported. Column 4 lists the percent of censuses reporting each species, or in other words, the percent of probability of seeing each species on an average winter hike during the Christmas season. This probability, of course, would not apply uniformly to all sections of the state. Column 5 indicates the percent of the total bird population which each species forms. For example, the table indicates that one-fourth of all Ohio winter birds are Crows, nearly one-fifth Tree Sparrows and that one bird of each 32 is a Cardinal. Column 6 ranks each species according to the number of individuals reported and No. 7, according to the number of times checked on census reports.

TABLE II.

Rank of Species Based on Both Numbers and Occurrences	Number Censuses and Number of Years on which Reported	Total Number Individuals Seen	Percent of Censuses Reporting	Percent of Total Bird Population	Ranking According to Individuals	Ranking According to Number Times Reported
1	*2*	*3*	*4*	*5*	*6*	*7*
1. Tree Sparrow	359—32	42,143	91.5	18.90	2	3
2. Cardinal	363—32	7,086	92.6	3.17	6	2
3. Song Sparrow	351—32	8,119	88.0	3.65	5	4
4. Crow	255—28	56,841	64.9	25.40	1	10
5. Slate-colored Junco	322—31	15,236	82.1	6.83	4	7
6. Downy Woodpecker	374—32	4,503	95.4	2.01	11	1
7. Tufted Titmouse	345—32	6,776	88.0	3.04	7	6
8. White-br. Nuthatch	349—32	4,695	89.0	2.10	10	5
9. Bob-white	209—30	6,767	53.3	3.03	8	13
10. Blue Jay	300—32	2,777	76.5	1.24	17	9
11. Black-capped Chickadee	179—32	5,050	45.6	2.37	9	16
12. Starling	98—11	15,782	25.0	7.08	3	26
13. Car. Chickadee	159—30	4,114	40.5	1.84	12	18
14. Hairy Woodpecker	302—31	1,096	77.0	.49	26	8
15. Northern Flicker	210—31	1,260	53.5	.56	24	12
16. Mourning Dove	114—27	2,896	29.1	1.30	16	22
17. Brown Creeper	191—26	1,198	48.7	.54	25	15
18. Goldfinch	156—29	1,593	39.8	.71	21	19
19. Red-bel. Woodpecker	211—31	945	53.8	.42	31	11
20. Robin	102—27	1,910	28.5	.85	20	23
21. Sparrow Hawk	205—30	725	52.3	.33	35	14
22. Carolina Wren	176—29	905	54.9	.40	32	17

TABLE II—(*Continued*)

Rank of Species Based on Both Numbers and Occurrences	Number Censuses and Number of Years on which Reported	Total Number Individuals Seen	Percent of Censuses Reporting	Percent of Total Bird Population	Ranking According to Individuals	Ranking According to Number Times Reported
1	*2*	*3*	*4*	*5*	*6*	*7*
23. Gold.-cr. Kinglet	143—27	1,024	36.4	.45	30	20
24. N. Horned Lark	57—17	2,950	14.5	1.33	15	38
25. Red-h. Woodpecker	98—24	1,028	25.5	.46	29	24
26. Herring Gull	63—18	1,365	16.1	.61	23	35
27. Meadowlark	91—21	886	23.2	.39	33	27
28. Black Duck	40—14	2,139	10.2	.95	18	44
29. Mallard	42—15	1,369	10.7	.61	22	43
30. Lesser Scaup	23—10	3,038	5.8	1.36	14	54
31. Red-tailed Hawk	122—30	208	30.8	.09	48	21
32. Towhee	76—20	512	19.3	.22	38	31
33. Bluebird	75—24	563	19.2	.25	37	32
34. Bonaparte's Gull	21—11	3,622	5.3	1.60	13	58
35. Pr. Horned Lark	40—19	1,032	10.2	.46	28	46
36. Red-winged Blackbird	37—14	1,081	9.4	.48	27	47
37. Winter Wren	85—21	280	21.6	.12	44	30
38. Marsh Hawk	88—16	218	22.4	.09	47	20
39. Screech Owl	98—21	157	25.0	.07	53	25
40. Kingfisher	90—20	162	22.9	.07	51	28
41. Bronzed Grackle	55—22	289	14.0	.13	43	39
42. Cowbird	27—14	739	6.80	.33	34	50
43. Am. Merganser	40—17	410	10.2	.18	40	45
44. R. N. Pheasant	61—18	190	15.8	.08	49	36
45. R. B. Nuthatch	51—26	252	13.0	.11	46	40
46. Snow Bunting	12— 6	2,114	4.0	.94	19	68
47. Cedar Waxwing	28—14	319	7.1	.14	41	49
48. Swamp Sparrow	25—11	304	6.3	.13	42	51
49. Red-should. Hawk	73—24	111	18.6	.04	60	33
50. Cooper's Hawk	64—21	100	16.3	.04	62	34
51. Purple Finch	24—13	254	6.1	.11	45	52
52. Pine Siskin	15—10	619	3.8	.27	36	63
53. Barred Owl	60—26	73	15.3	.03	64	37
54. Ruffed Grouse	51—25	103	13.0	.04	61	41
55. Sharp-shin. Hawk	45—23	63	10.1	.02	67	42
56. Golden-eye	21—12	149	5.3	.06	54	57
57. Rusty Blackbird	15— 9	147	3.8	.06	56	62
58. Gr't. Horned Owl	36—20	45	9.4	.01	71	48
59. Short-eared Owl	18—12	70	4.6	.03	66	59
60. Ring-billed Gull	9— 6	175	2.3	.07	50	78
61. Rough-legged Hawk	24—13	36	6.1	.01	76	53
62. Hooded Merganser	10— 7	147	2.5	.06	55	76
63. Redpoll	10— 7	123	2.5	.05	58	73
64. Killdeer	14— 8	59	3.5	.02	68	65
65. Y. B. Sapsucker	21—16	27	5.3	.01	80	56
66. Mockingbird	23—12	25	5.8	.01	82	55
67. American Coot	7— 4	160	1.8	.07	52	86
68. Barn Owl	17— 9	28	4.3	.01	79	60
69. Pileated Woodpecker	16—10	26	4.1	.01	81	61
70. Canada Goose	7— 5	136	1.8	.06	57	87
71. Field Sparrow	11— 9	41	2.8	.01	74	72
72. Myrtle Warbler	11— 7	37	2.8	.01	75	71
73. W. T. Sparrow	9— 8	53	2.3	.02	69	77
74. Pintail	12— 6	33	3.1	.01	78	69

TABLE II—(*Continued*)

Rank of Species Based on Both Numbers and Occurrences	Number Censuses and Number of Years on which Reported	Total Number Individuals Seen	Percent of Censuses Reporting	Percent of Total Bird Population	Ranking According to Individuals	Ranking According to Number Times Reported
1	*2*	*3*	*4*	*5*	*6*	*7*
75. Hungarian Partridge...	7— 6	87	1.8	.03	63	85
76. Turkey Vulture.....	12—10	26	3.1	.01	83	67
77. Bewick's Wren.....	15—12	21	3.8	.009	87	64
78. Ruddy Duck.....	5— 3	122	1.2	.05	59	92
79. Bald Eagle.....	12—11	24	3.1	.01	84	70
80. Red Crossbill.....	6— 4	72	1.5	.03	65	90
81. Goshawk.....	14— 7	15	3.5	.006	91	66
82. Pied-billed Grebe.....	7— 6	8	1.8	.003	78	80
83. American Pipit.....	1— 1	500	0.2	.220	39	121
84. Canvasback.....	7— 5	43	1.8	.01	73	88
85. Red-br. Merganser.....	5— 3	50	1.2	.02	70	93
86. Horned Grebe.....	10— 6	16	2.5	.007	90	75
87. Fox Sparrow.....	8— 6	19	2.0	.008	88	79
88. Northern Shrike.....	10— 7	13	2.5	.005	95	74
89. Ring-necked Duck.....	4— 3	35	1.0	.015	77	95
90. Lapland Longspur.....	7— 6	15	1.8	.006	92	81
91. Baldpate.....	7— 3	24	1.8	.010	85	89
92. Bufflehead.....	7— 5	13	1.8	.005	94	83
93. Hermit Thrush.....	7— 5	7	1.8	.003	99	82
94. Shoveller.....	4— 2	19	1.0	.008	89	94
95. Black Vulture.....	3— 3	44	0.7	.018	72	105
96. Great Blue Heron.....	6— 4	8	1.5	.003	97	91
97. Bohemian Waxwing....	3— 3	14	0.7	.006	93	104
98. Redhead Duck.....	3— 2	7	0.7	.003	101	99
99. Long-eared Owl.....	4— 4	5	1.0	.002	103	97
100. Green-winged Teal.....	2— 1	21	0.5	.009	86	114
101. Common Loon.....	4— 2	4	1.0	.002	106	96
102. Gadwall.....	3— 2	4	0.7	.002	105	103
103. Palm Warbler.....	3— 3	4	0.7	.002	107	101
104. Pigeon Hawk.....	3— 3	3	0.7	.001	112	100
105. Migrant Shrike.....	3— 3	3	0.7	.001	111	102
106. Wilson's Snipe.....	3— 3	3	0.7	.001	115	98
107. Old-squaw.....	2— 2	7	0.5	.003	100	113
108. White-w'g'd Crossbill..	1— 1	11	0.2	.004	96	118
109. B. Cr. Night Heron....	3— 2	3	0.7	.001	110	106
110. W. Cr. Sparrow.....	2— 2	4	0.5	.002	109	118
111. Saw-whet Owl.....	2— 2	3	0.5	.001	114	110
112. Ruby-cr. Kinglet.....	2— 2	3	0.5	.001	113	111
113. Prairie Marsh Wren....	1— 1	4	0.2	.002	108	120
114. Wood Thrush.....	2— 2	2	0.5	.0009	122	107
115. Snowy Owl.....	2— 1	2	0.5	.0009	120	109
116. Phoebe.....	2— 2	2	0.5	.0009	118	112
117. D. C. Cormorant.....	2— 2	2	0.5	.0009	116	115
118. Evening Grosbeak.....	1— 1	5	0.2	.002	102	129
119. Woodcock.....	2— 2	2	0.5	.0009	121	116
120. Holboell's Grebe.....	1— 1	2	0.2	.0009	117	126
121. Lesser Snow Goose.....	1— 1	2	0.2	.0009	119	124
122. American Bittern.....	1— 1	4	0.2	.002	104	132
123. White-winged Scoter...	1— 1	1	0.2	.0005	132	117
124. Vesper Sparrow.....	1— 1	1	0.2	.0005	131	119
125. Orange-cr. Warbler....	1— 1	1	0.2	.0005	129	123
126. Pine Grosbeak.....	1— 1	1	0.2	.0005	130	122

TABLE II—(*Continued*)

Rank of Species Based on Both Numbers and Occurrences	Number Censuses and Number of Years on which Reported	Total Number Individuals Seen	Percent of Censuses Reporting	Percent of Total Bird Population	Ranking According to Individuals	Ranking According to Number Times Reported
1	*2*	*3*	*4*	*5*	*6*	*7*
127. Indigo Bunting	1— 1	1	0.2	.0005	128	125
128. Golden Eagle	1— 1	1	0.2	.0005	127	127
129. Franklin's Gull	1— 1	1	0.2	.0005	126	128
130. Brown Thrasher	1— 1	1	0.2	.0005	123	132
131. Duck Hawk	1— 1	1	0.2	.0005	124	131
132. Chipping Sparrow	1— 1	1	0.2	.0005	124	130

Summary—Thirty-two Years, 1900–1931.

Total number of individuals seen	222,825
Total number of species	133
Total number of censuses taken	392
Total number of census co-operators	934
Average number species seen on each census, 1900–1931	18.8
Average number species seen on each census, 1922–1931	25.0
Average number individuals seen on each census (32 years)	569
Number of counties (out of 88) in which censuses were taken	49
Number of localities in which censuses were taken	79

The following comparisons should be of interest. The first ranking 25 species are given as follows:

Rank Based on Number of Individuals	Rank Based on Number of Times Reported	Rank Based on Both the Numbers of Individuals and Times Reported
1. Crow	1. Downy Woodpecker	1. Tree Sparrow
2. Tree Sparrow	2. Cardinal	2. Cardinal
3. Starling	3. Tree Sparrow	3. Song Sparrow
4. Junco	4. Song Sparrow	4. Crow
5. Song Sparrow	5. W. B. Nuthatch	5. Junco
6. Cardinal	6. Tufted Titmouse	6. Downy Woodpecker
7. Tufted Titmouse	7. Junco	7. Tufted Titmouse
8. Bob-white	8. Hairy Woodpecker	8. W. B. Nuthatch
9. B. Cap. Chickadee	9. Blue Jay	9. Bob-white
10. W. B. Nuthatch	10. Crow	10. Blue Jay
11. Downy Woodpecker	11. Red-bel. Woodpecker	11. B. Cap. Chickadee
12. Car. Chickadee	12. Northern Flicker	12. Starling
13. Bonaparte's Gull	13. Bob-white	13. Car. Chickadee
14. Lesser Scaup	14. Sparrow Hawk	14. Hairy Woodpecker
15. N. Horned Lark	15. Brown Creeper	15. Northern Flicker
16. Mourning Dove	16. B. Cap. Chickadee	16. Mourning Dove
17. Blue Jay	17. Carolina Wren	17. Brown Creeper
18. Black Duck	18. Car. Chickadee	18. Goldfinch
19. Snow Bunting	19. Goldfinch	19. Red-wgd. Blackbird
20. Robin	20. G. C. Kinglet	20. Robin
21. Goldfinch	21. Red-tailed Hawk	21. Sparrow Hawk
22. Mallard	22. Mourning Dove	22. Carolina Wren
23. Herring Gull	23. Robin	23. G. C. Kinglet
24. Northern Flicker	24. Red-headed Woodpecker	24. N. Horned Lark
25. Brown Creeper	25. Screech Owl	25. Red-headed Woodpecker

Mr. Sidney R. Esten (1931), in compiling the 133 Indiana Christmas Censuses from 28 counties for the years 1900 to 1929, found that 34,299 individuals and 74 species were recorded. The average number of individuals seen on each hike was 258 and of species, 16. The first ranking 25 species (numerical ranking) were as follows: Junco, Tree Sparrow, Crow, Tufted Titmouse, Song Sparrow, Chickadee (both species), Cardinal, Downy Woodpecker, Blue Jay, White-breasted Nuthatch, Mourning Dove, Bob-white, Goldfinch, Bronzed Grackle, Flicker, Prairie Horned Lark, Brown Creeper, Horned Lark, Bluebird, Red-headed Woodpecker, Hairy Woodpecker, Meadowlark, Robin, Carolina Wren, and Lapland Longspur. Indiana censuses indicated increases of Starlings, Crows, Cardinals, Mallards, and Herring Gulls, and decreases of Song Sparrows, Chickadees, Tufted Titmice, Blue Jays, and Downy Woodpeckers.

In Ohio, a number of factors make it difficult to conclude definitely as to any pronounced increases or decreases of the various bird species enumerated and this aspect would be too lengthy to discuss in the present paper. It is planned to continue to collect abundance data on each species from various sources, and to publish the same after a longer period of comparative studies.

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Treatise on Sedimentation (second edition), by W. H. Twenhofel. 960 pp., 121 ill. Baltimore, the Williams and Wilkins Co., 1932. $8.00.